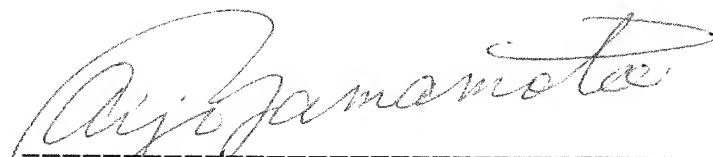


TRANSLATION

I, Aiji Yamamoto, residing at 1-13-16, Shibayama, Funabashi-shi, Chiba-ken, Japan, state:

that I know well both the Japanese and English languages; that I translated, from Japanese into English, Japanese Patent Application No. 2003-101346, filed on April 4, 2003; and that the attached English translation is a true and accurate translation to the best of my knowledge and belief.

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DESCRIPTION

[Title of the Invention]

TOTAL INTERNAL REFLECTION
FLUORESCENCE MICROSCOPE

[What is Claimed is:]

[Claim 1]

A total internal reflection fluorescence microscope characterized by comprising:

an objective lens which takes in light from a specimen;

an observation optical path via which the light taken into the objective lens is condensed onto an image pick-up device via an image forming lens;

a condenser lens for transmission illuminating which is disposed in a position facing the objective lens via the specimen and which has a numerical aperture that makes possible total internal reflection illumination;

a reflective mirror which is movably disposed in the vicinity of an outermost part of an optical path of a transmitted illuminative light, closer to an optical source side rather than the condenser lens and which reflects a laser beam to introduce the laser beam on the condenser lens side;

a laser introductory optical path which allows the laser beam to be incident upon the reflective mirror from a direction crossing the optical path of the transmitted illuminative light substantially at right angles; and

moving means for moving the reflective mirror in a direction parallel to the laser introductory optical path, and

the laser introductory optical path comprises:

a fiber which transmits the laser beam output from a

laser oscillation unit; and

a projection lens unit which converts a divergent ray emitted from an emission end of the fiber into a convergent ray to condense the ray in the vicinity of a front focal position of the condenser lens via the reflective mirror.

[Claim 2]

The total internal reflection fluorescence microscope according to claim 1, characterized in that the laser introductory optical path further comprises a conversion lens unit which is integrally and detachably inserted between the emission end of the fiber and the projection lens unit, and

the conversion lens unit converts an incidence NA of the laser beam incident upon a condensing position without changing the condensing position of the laser beam by being integrally and detachably inserted into the laser introductory optical path.

[Claim 3]

The total internal reflection fluorescence microscope according to claim 2, characterized in that one of a plurality of objective lenses having different observation magnifications is selectively disposed on the observation optical path, and

the total internal reflection fluorescence microscope further comprises control means for controlling inserting/detaching of the conversion lens unit into/from the laser introductory optical path in accordance with an observation magnification of the objective lens.

[Claim 4]

The total internal reflection fluorescence microscope

according to claim 1, characterized in that the laser introductory optical path further comprises a zoom lens unit consisting of a lens group each of which is movably disposed in an optical axis direction between the fiber emission end and the projection lens unit, and

the zoom lens unit continuously converts an incidence NA of the laser beam incident upon a condensing position without changing the condensing position of the laser beam by converting a positional relation of the lens group constituting the zoom lens unit in each optical axis direction.

[Claim 5]

The total internal reflection fluorescence microscope according to claim 4, characterized in that one of a plurality of objective lenses having different observation magnifications is selectively disposed on the observation optical path, and

the total internal reflection fluorescence microscope further comprises control means for determining a relative positional relation of the lens group disposed in the zoom lens unit in each optical axis direction in accordance with an observation magnification of the objective lens.

[Claim 6]

A total internal reflection fluorescence microscope characterized by comprising:

an objective lens which takes in light from a specimen;
an observation optical path via which the light taken into the objective lens is condensed onto an image pick-up device via an image forming lens;

single or plural dividing means disposed on optional

position on the observation optical path to divide the observation optical path depending on optical characteristics;

a condenser lens for transmission illuminating which is disposed in a position facing the objective lens via the specimen and which has a numerical aperture that makes possible total internal reflection illumination;

a reflective mirror which is movably disposed in the vicinity of an outermost part of an optical path of a transmitted illuminative light, closer to an optical source side rather than the condenser lens and which reflects a laser beam to introduce the laser beam on the condenser lens side;

a laser introductory optical path which allows the laser beam to be incident upon the reflective mirror from a direction crossing the optical path of the transmitted illuminative light substantially at right angles; and

moving means for moving the reflective mirror in a direction parallel to the laser introductory optical path,

the laser introductory optical path comprising:

a fiber which transmits the laser beam output from a laser oscillation unit; and

a projection lens unit which converts a divergent ray emitted from an emission end of the fiber into a convergent ray to condense the ray in the vicinity of a front focal position of the condenser lens via the reflective mirror, and

a plurality of laser introduction sections each composed of a set of the reflective mirror, moving means and laser introductory optical path are disposed radially centering on the transmitted illuminative light path and in a direction

crossing an optical axis of the transmitted illuminative light path substantially at right angles.

[Claim 7]

The total internal reflection fluorescence microscope according to claim 6, characterized by further comprising:

an optical path length adjustment section which is disposed on a plurality of observation optical paths divided by the single or plural optical dividing means and extends/contracts each optical path length.

[Claim 8]

The total internal reflection fluorescence microscope according to claim 7, characterized by further comprising:

a calculation section which calculates/processes an image from each image pick-up device and an extension/contraction of the optical path length by the optical path length adjustment section.

[Claim 9]

The total internal reflection fluorescence microscope according to claim 7 or 8, characterized in that each of the plurality of laser instruction sections further comprises:

a shutter to select the introducing and blocking of the laser beam; and

introduced laser selecting means for controlling the opening/closing of each shutter to sort out the introduced laser beam.

[Claim 10]

The total internal reflection fluorescence microscope according to claim 9, characterized in that the plurality of

laser introduction sections comprise: at least two laser introduction sections which output the laser beams having an equal wavelength.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

The present invention relates to a total internal reflection fluorescence microscope for performing fluorescence observation by use of an evanescent light generated by total internal reflection illumination.

[0002]

[Prior Art]

In recent years, a total internal reflection fluorescence microscopy (TIRFM) has attracted attentions as a microscope for fluorescence observation of a living thing. In this TIRFM, an illuminative light is totally reflected by a boundary surface between a cover glass and a specimen, and a fluorescent substance is excited using a light called an evanescent light which leaks into a small region having a size of several hundreds nm or less on a specimen side. In this TIRFM, only the fluorescence of the small region in the vicinity of the cover glass is observed. An observed image of TIRFM provides a very dark background. Accordingly, it is possible to observe fluorescence having a high contrast and faint fluorescence.

[0003]

Additionally, in a site of biological research using a total internal reflection fluorescence microscopy (hereinafter referred to as TIRFM), there are a case where a shallow plane

is to be observed with good contrast in the vicinity of the boundary surface between the cover glass and the specimen, and a case where the evanescent light is extended to a certain degree of depth to observe a broad range. Therefore, it is desirable to change a leak-out depth of the evanescent light in accordance with the specimen.

[0004]

The leak-out depth of the evanescent light from the boundary surface is described, for example, in D. Axelrod's document "Total Internal Reflection Fluorescence at Biological Surfaces". Accordingly, the following equation is established.

$$d = \lambda / 4\pi \sqrt{(n_1^2 \cdot \sin \theta_1^2 - n_2^2)} \quad \dots (1),$$

where d denotes the leak-out depth of the evanescent light, λ denotes a wavelength of the light, n_1 denotes a refractive index on the incidence side, θ_1 denotes an incidence angle, and n_2 denotes a refractive index on an emission side.

Therefore, when the incidence angle of the illuminative light with respect to the boundary surface, that is, an inclination angle of the illuminative light with respect to a normal to the boundary surface increases, the leak-out depth of the evanescent light becomes shallow. In actual TIRFM, a laser beam having a high coherent property is used, and the incidence angle of the illuminative light is adjusted. Accordingly, the incidence angle of the laser beam onto the boundary surface changes, and the leak-out depth of the evanescent light is adjusted.

[0005]

FIG. 16 shows a constitution of a TIRFM disclosed in Pat. Document 1. Here, an objective lens 104 having a numerical aperture with which total internal reflection illumination is possible is used. A mirror 107 which reflects a laser beam 106 for use as an illuminative light onto an objective lens 104 side is moved in a direction crossing an optical axis direction of the objective lens 104 at right angles, as illustrated in the order of (a) → (b) → (c) in FIG. 16. A position of the laser beam 106 incident upon the objective lens 104 moves in a direction distant from an optical axis of the objective lens 104. By the movement of the incidence position of the laser beam, the incidence angle of the laser beam 106 emitted toward the boundary surface between a cover glass 102 and a specimen 101 from the objective lens 104 changes. The laser beam 106 emitted from the objective lens 104 is totally reflected by the boundary surface between the cover glass 102 and specimen 101 via an immersion oil 102 as shown in FIG. 16(c).

[0006]

FIG. 17 shows a constitution of TIRFM disclosed in Pat. Document 2. In the TIRFM, the laser beam 106 is incident upon a side surface 261a of a point ball lens 261 of a condenser lens for transmission illuminating and the total internal reflection illumination is possible.

[0007]

A laser illuminating device 150 itself is rotatable with respect to a microscope main body 160 with the TIRFM constitution. The laser illuminating device 150 rotates

centering on an intersection of the boundary surface of the cover glass 102 and specimen 101 and the observation optical axis. Accordingly, the laser beam 106 changes its incidence angle with respect to the boundary surface between the cover glass 102 and specimen 101.

[0008]

[Pat. Document 1]

Japanese Patent No. 3093145 (refer to paragraphs [0002] - [0021] and [FIG. 1] - [FIG. 4])

[0009]

[Pat. Document 2]

Jpn. Pat. Appln. Publication No. 2001-013413 (refer to paragraphs [0014] - [0051] and [FIG. 1] - [FIG. 3])

[0010]

[Objects of the Invention]

Meanwhile, in order to totally reflect the laser beam 106 as the illuminative light upon the boundary surface of the cover glass 102 and specimen 101, the incidence angle of the laser beam 106 needs to be inclined by a critical angle or more, at which the total internal reflection occurs. Here, assuming that a refractive index on a cover glass 102 side via the boundary surface between the cover glass 102 and specimen 101 is n_1 , and a refractive index on a specimen 101 side is n_2 , a critical angle θ_c is represented by the following equation (2).

$$\sin\theta_c = n_2/n_1 \quad \dots (2)$$

Therefore, conditions of the incidence angle θ_1 for

realizing the total internal reflection illumination is represented by the following equation (3).

$$n_1 \cdot \sin\theta_1 > n_2 \quad \dots (3)$$

On the other hand, to incline an incident light of the laser beam 106 passing through the objective lens 104 as in Pat. Document 1 shown in FIG. 16, a maximum incidence angle θ_{max} that can be set depends on the numerical aperture (NA) of the objective lens 104, and is represented by the following equation (4).

$$n_1 \cdot \sin\theta_{max} = NA \quad \dots (4)$$

Therefore, for the conditions for realizing the total internal reflection illumination, the NA of the objective lens needs to be larger than the refractive index n_2 on the specimen side.

[0011]

In general, a refractive index of a living cell is about 1.37 to 1.38. The NA of the objective lens for use needs to be about 1.4 at minimum.

At present, a magnification of the objective lens having an NA of 1.4 or more is limited to a high magnification of 60 times or more. To realize a high NA by the objective lens having a low magnification, an effective diameter of the objective lens needs to be increased. However, it is difficult to increase the effective diameter of the objective lens while keeping a standard diameter of an attaching screw of the objective lens. Therefore, in the TIRFM shown in FIG. 16, total internal reflection fluorescence observation at a

magnification of about 20 or 40 times is impossible.

[0012]

In contrast, as disclosed in Pat. Document 2 shown in FIG. 17, when the laser beam 106 is incident from a condenser lens for transmission illuminating side, an illuminative range can be set without depending on the objective lens 104. Accordingly, the total internal reflection fluorescence observation using the objective lens having a low magnification is possible.

[0013]

However, in the above constitution, the laser illuminating device 150 is disposed right beside the point ball lens 261 of the condenser lens for transmission illuminating. Additionally, the laser illuminating device 150 itself needs to be rotated. Therefore, a considerable space is necessary including a holding section of a rotary mechanism and a space of a track of the rotating laser illuminating device 150. Consequently, a space in which the specimen is laid is compressed. It is supposed that operation properties are remarkably impaired.

[0014]

An irradiation range of the laser beam is set in such a manner that an observation range of the objective lens having the low magnification can be illuminated. Then, in the observation with the objective lens having the high magnification, only a part of the irradiation range of the laser beam is observed. Therefore, the laser beam with which another part is irradiated is useless.

An energy density of the laser beam on the surface of the specimen is in inverse proportion to an irradiation area. In the observation with the objective lens having the high magnification, the irradiation range of the laser beam is condensed so as to illuminate only a range required for the observation, and the energy density of the laser beam is preferably enhanced.

[0015]

Especially, there is an experiment for the purpose of detection of very weak fluorescence such as a single molecule. In this experiment, the irradiation energy density of the laser beam is required to be as high as possible. On the other hand, in the TIRFM, the leak-out depth of the evanescent light is changeable. In recent years, the TIRFM has been spread in the site of the biological research. Furthermore, there has started to be a demand for the simultaneous illuminating of a plurality of optional wavelengths in optional depths.

[0016]

This background has the following actual circumstances. Improvement of fluorescent protein such as GFP has been advanced, and it becomes easy to observe a dynamic state or a function of the living cell with multicolored fluorescence. Moreover, as seen also from Axelrod formula (equation (1)), the leak-out depth of the evanescent light depends also on the wavelength of the light. Therefore, there is a principle problem that a range to be observed differs, when the wavelength differs even at the equal laser beam incidence angle. There is also a realistic problem that a depth position

of a tissue in a cell corresponding to each wavelength differs.

[0017]

In the conventional TIRFM, it is possible to switch the incidence angle or the wavelength of the laser beam at a high speed using mechanical means or electric driving means such as a motor. However, in cases where a simultaneous property in a strict meaning is required such as a case where a fast phenomenon is traced, there is a restriction on a high-speed switch. In this case, it is necessary to simultaneously illuminate introductory portions of the laser beams disposed in a plurality of places.

[0018]

However, in the TIRFM disclosed in Pat. Document 1 shown in FIG. 16, a dichroic mirror is used in order to reflect the illuminative light on an objective lens side and to transmit the fluorescence on an observation side.

[0019]

Additionally, when there are a plurality of wavelengths to be illuminated, the dichroic mirror needs to have wavelength characteristics of the corresponding multi-band. The dichroic mirror of the multi-band has a high difficulty in manufacturing, and is expensive. Furthermore, the dichroic mirror of the multi band has a bad separation level of the wavelength, and brightness and SN ratio of a fluorescent image are deteriorated. When the illuminative wavelengths are to be further increased halfway, the dichroic mirror needs to be newly prepared again.

[0020]

On the other hand, to prevent the dichroic mirror from being used, as shown in FIG. 18, it is possible to dispose a total internal reflection mirror 108a in a position of an outermost portion of a pupil of the objective lens 104. However, it is necessary to dispose another total internal reflection mirror 108b also in the outermost portion of the pupil of the objective lens 104 on an opposite side in order to prevent the laser beam 106 totally reflected by the boundary surface between the cover glass and the specimen 101 from passing on an observation side.

[0021]

Therefore, a considerable part of the pupil of the objective lens 104, which should have been originally used 100% for observation, is lost by the respective total internal reflection mirrors 108a, 108b. Therefore, a capability of the objective lens is deteriorated.

[0022]

In the TIRFM disclosed in Pat. Document 2 shown in FIG. 17, as described above, the laser illuminating device 150 is disposed right beside the point ball lens 261 of the condenser lens for transmission illuminating, and additionally the laser illuminating device 150 itself needs to be rotated. Therefore, a considerable space is required including the space of the track of the holding section of the rotation mechanism or the rotating laser illuminating device 150. In this constitution, when the laser illuminating device including an emission angle adjustment section of independent laser beams is

to be disposed, two laser illuminating devices at maximum can be disposed on opposite sides of the condenser lens for transmission illuminating.

[0023]

Moreover, each TIRFM in prior art has a common problem. There is a case where a plurality of wavelengths are observed by the use of the evanescent lights having different depths. For example, when a shallow region is observed with B excitation, and a deep region is observed with G excitation, the B and G excitations can be simultaneously observed only in the shallow region. In this case, the image of the G excitation can only be observed as a defocused background image. Therefore, for example, the whole cell film is dyed, and the image of the TIRFM in the deep region can be used only in limited applications such as grasping of an approximate size of the cell.

[0024]

While the objective lens is fixed, the surfaces in the different depths can be simultaneously observed. Then, the application of multi-wavelength TIRFM can further be broadened to simultaneous observation of forms of small organs in the vicinity of the cell film and inside the cell.

[0025]

The present invention has been made in view of the requirements described above, and it is an object of the invention to provide a total internal reflection fluorescence microscope which enables an object lens having a magnification of lower than 60 times to achieve total internal reflection

fluorescence observation, and which is compact and does not compress the operation space of a specimen with a superior operation property.

[0026]

It is also an object of the present invention to provide a total internal reflection fluorescence microscope which can convert the laser beam irradiation range in accordance with the observation range of an objective lens having different magnifications.

Further, it is an object of the invention to provide a total internal reflection fluorescence microscope in which a plurality of laser beam introduction sections are provided, allowing evanescent lights having different wavelengths and leak-out depths to be simultaneously irradiated.

[0027]

Moreover, it is an object of the invention to provide a total internal reflection fluorescence microscope in which a combination of evanescent lights having different depths is used for illumination, which allows surfaces having different depths on the specimen to be simultaneously observed with a high contrast.

[0028]

[Means for Achieving the Objects]

To achieve the aforementioned objects of the present invention, there is provided a total internal reflection fluorescence microscope as described in claim 1, which is characterized by comprising: an objective lens which takes in light from a specimen; an observation optical path via which

the light taken into the objective lens is condensed onto an image pick-up device via an image forming lens; a condenser lens for transmission illuminating which is disposed in a position facing the objective lens via the specimen and which has a numerical aperture that makes possible total internal reflection illumination; a reflective mirror which is movably disposed in the vicinity of an outermost part of an optical path of a transmitted illuminative light, closer to an optical source side rather than the condenser lens and which reflects a laser beam to introduce the laser beam on the condenser lens side; a laser introductory optical path which allows the laser beam to be incident upon the reflective mirror from a direction crossing the optical path of the transmitted illuminative light substantially at right angles; and a moving means for moving the reflective mirror in a direction parallel to the laser introductory optical path. The laser introductory optical path comprises: a fiber which transmits the laser beam output from a laser oscillation unit; and a projection lens unit which converts a divergent ray emitted from an emission end of the fiber into a convergent ray to condense the ray in the vicinity of a front focal position of the condenser lens via the reflective mirror.

[0029]

According to claim 2 of the present invention, there is provided a total internal reflection fluorescence microscope, which is characterized in that the laser introductory optical path set forth in claim 1 further comprises a conversion lens unit which is integrally and detachably inserted between the

emission end of the fiber and the projection lens unit. The conversion lens unit converts an incidence NA of the laser beam incident upon a condensing position without changing the condensing position of the laser beam by being integrally and detachably inserted into the laser introductory optical path.

[0030]

According to claim 3 of the present invention, there is provided a total internal reflection fluorescence microscope, which is characterized in that the objective lens as set forth in claim 2 consists of plural objective lenses having different observation magnifications, one of which being selectively disposed on the observation optical path, and the total internal reflection fluorescence microscope further comprises a control means for controlling inserting/detaching of the conversion lens unit into/from the laser introductory optical path in accordance with an observation magnification of the objective lens.

[0031]

According to claim 4 of the present invention, there is provided a total internal reflection fluorescence microscope, which is characterized in that the laser introductory optical path set forth in claim 1 further comprises a zoom lens unit consisting of a lens group each of which is movably disposed in an optical axis direction between the fiber emission end and the projection lens unit. The zoom lens unit continuously converts an incidence NA of the laser beam incident upon a condensing position without changing the condensing position of the laser beam by converting a positional relation of the lens

group in each optical axis direction.

[0032]

According to claim 5 of the present invention, there is provided a total internal reflection fluorescence microscope, which is characterized in that the objective lens set forth in claim 4 consists of plural objective lenses having different observation magnifications, one of which being selectively disposed on the observation optical path, and the total internal reflection fluorescence microscope further comprises a control means for determining a relative positional relation of the lens group disposed in the zoom lens unit in each optical axis direction in accordance with an observation magnification of the objective lens.

[0033]

According to claim 6 of the present invention, there is provided a total internal reflection fluorescence microscope, which is characterized by comprising: an objective lens which takes in light from a specimen; an observation optical path via which the light taken into the objective lens is condensed onto an image pick-up device via an image forming lens; a single or plural dividing means which is (are) disposed on optional position on the observation optical path to divide the observation optical path depending on optical characteristics; a condenser lens for transmission illuminating which is disposed in a position facing the objective lens via the specimen and which has a numerical aperture that makes possible total internal reflection illumination; a reflective mirror which is movably disposed in the vicinity of an outermost part

of an optical path of a transmitted illuminative light, closer to an optical source side rather than the condenser lens and which reflects a laser beam to introduce the laser beam on the condenser lens side; a laser introductory optical path which allows the laser beam to be incident upon the reflective mirror from a direction crossing the optical path of the transmitted illuminative light substantially at right angles; and a moving means for moving the reflective mirror in a direction parallel to the laser introductory optical path. The laser introductory optical path comprises: a fiber which transmits the laser beam output from a laser oscillation unit; and a projection lens unit which converts a divergent ray emitted from an emission end of the fiber into a convergent ray to condense the ray in the vicinity of a front focal position of the condenser lens via the reflective mirror, and a plurality of laser introduction sections each composed of a set of the reflective mirror, moving means and laser introductory optical path are disposed radially centering on the transmitted illuminative light path and in a direction crossing an optical axis of the transmitted illuminative light path substantially at right angles.

[0034]

According to claim 7 of the present invention, there is provided a total internal reflection fluorescence microscope, which is characterized by further comprising: an optical path length adjustment section which is disposed on a plurality of observation optical paths divided by the single or plural optical dividing means set forth in claim 6 and

extends/contracts each optical path length.

[0035]

According to claim 8 of the present invention, there is provided a total internal reflection fluorescence microscope set forth in claim 7, which is characterized by further comprising: a calculation section which calculates/processes an image from each image pick-up device and an extension/contraction of the optical path length by the optical path length adjustment section.

[0036]

According to claim 9 of the present invention, there is provided a total internal reflection fluorescence microscope, which is characterized in that each of the plurality of laser introduction sections as set forth in claim 7 or 8 further comprises: a shutter to select the introducing and blocking of the laser beam; and an introduced laser selecting means for controlling the opening/closing of each shutter to sort out the introduced laser beam.

[0037]

According to claim 10 of the present invention, there is provided a total internal reflection fluorescence microscope, which is characterized in that the plurality of laser introduction sections set forth in claim 9 comprise: at least two laser introduction sections which output the laser beams having an equal wavelength.

[0038]

According to the total internal reflection fluorescence microscope described in claim 1 of the present invention, the

condenser lens has a numerical aperture with which total internal reflection illumination is possible. Therefore, fluorescence observation by the total internal reflection illumination is possible regardless of the numerical aperture or the magnification of the objective lens. The laser introductory optical path is configured to be farther than the condenser lens from the specimen. Accordingly, a space in the vicinity of the specimen is not compressed. In addition, the laser introduction section has a simple structure and also has a narrow operation range. Therefore, the TIRFM itself can be compact.

[0039]

According to the total internal reflection fluorescence microscope described in claim 2 of the present invention, the conversion lens unit is detachably inserted into the laser introductory optical path. Accordingly, the incidence NA of the laser beam condensed in the vicinity of the front focal position of the condenser lens is converted. As a result, the irradiation range of the laser beam on the specimen, that is, the energy density of the laser beam can be converted.

[0040]

According to the total internal reflection fluorescence microscope described in claim 3 of the present invention, when the observation magnification of the objective lens is switched, the control means selects the switching of the laser beam irradiation range which corresponds to the observation range in cooperation of the observation magnification. Accordingly, the conversion lens unit is inserted/detached

into/from the laser introductory optical path.

[0041]

According to the total internal reflection fluorescence microscope described in claim 4 of the present invention, the incidence NA of the laser beam condensed in the vicinity of the front focal position of the condenser lens is continuously converted. As a result, the irradiation range of the laser beam on the specimen, that is, the energy density of the laser beam can be continuously converted.

[0042]

According to the total internal reflection fluorescence microscope described in claim 5 of the present invention, when the observation magnification of the objective lens is optionally switched, the laser beam irradiation range corresponding with the observation range of the objective lens can be freely set and switched in cooperation with the magnification of the selected objective lens.

[0043]

According to the total internal reflection fluorescence microscope described in claim 6 of the present invention, the condenser lens has a numerical aperture with which total internal reflection illumination is possible. Therefore, fluorescence observation by the total internal reflection illumination is possible regardless of the numerical aperture or the magnification of the objective lens. The laser introductory optical path is configured to be farther than the condenser lens from the specimen. Accordingly, a space in the vicinity of the specimen is not compressed. In addition, the

laser introduction section has a simple structure and also has a narrow operation range. Therefore, the TIRFM itself can be compact. Further, a plurality of laser introduction sections can easily be added. The leak-out depths of the evanescent light can be independently set and it is possible to simultaneously irradiate the specimen with the laser beam having a plurality of wavelengths.

[0044]

According to the total internal reflection fluorescence microscope described in claim 7 of the present invention, the surfaces having different depths on the specimen can be simultaneously observed with a high contrast in total internal reflection illumination while the focal position of the objective lens is fixed.

[0045]

According to the total internal reflection fluorescence microscope described in claim 8 of the present invention, an image from each image pick-up device and an extension/contraction of the optical path length by the optical path length adjustment section can be adjusted through the calculation/processing by the calculation section.

[0046]

According to the total internal reflection fluorescence microscope described in claim 9 of the present invention, the laser beams introduced into the specimen can be selected by the opening/closing of respective shutters.

According to the total internal reflection fluorescence microscope described in claim 10 of the present invention, for

the laser introduction sections in which the wavelengths of the laser beams are set to be equal, the respective laser beams are alternately introduced into the specimen by the opening/closing the shutters while the leak-out depth of the fluorescence is changed. As a result, when the observed images by the difference in the leak-out depth of the fluorescence by the same fluorescent substance are compared, it is possible to specify the depth of the generation source of the fluorescence.

[0047]

[Embodiments of the Invention]

(First embodiment) FIG. 1 is a constitution diagram showing a first embodiment of the present invention.

In FIG. 1, reference numeral 1 represents a condenser lens of the microscope. Reference numeral 2 represents an immersion oil, which is dotted/attached to a tip of the condenser lens 1. Reference numeral 3 represents a slide glass for observation. Reference numeral 4 represents a specimen disposed on the slide glass 3. Further, reference numeral 5 represents a cover glass which is disposed on the opposite side of the slide glass 3 with the specimen 4 sandwiched therebetween. Reference numeral 6 represents an objective lens for observing the specimen 4 through the cover glass 5.

[0048]

Here, a numerical aperture (NA) of the condenser lens 1 is designed to be larger than a refractive index of the specimen 4. That is, assuming that a refractive index of the immersion oil 2 or the slide glass 3 is n_1 , and a refractive index of the specimen 4 is n_2 , the following relation is

established from above equations (3) and (4):

$$NA = n_1 \cdot \sin\theta_{max} > n_2 \quad \dots (5),$$

where θ_{max} corresponds to a maximum incidence angle at which the incidence is possible through the immersion oil 2 and slide glass 3 from the condenser lens 1. In general, the refractive index of the living cell is about 1.37 to 1.38. Accordingly, the numerical aperture of the condenser lens 1 has a value larger than the refractive index of the living cell of 1.37 to 1.38, and concretely has a value of about 1.65 to 1.45.

[0049]

Further, reference numeral 7 represents a transmitted illuminative light source, and 8 represents a collector lens which introduces the illuminative light output from the transmitted illuminative light source 7 into the condenser lens 1. Reference numeral 9 represents a reflective mirror which is disposed on a transmitted illuminative light path T extending between the condenser lens 1 and the transmitted illuminative light source 7.

[0050]

The reflective mirror 9 is disposed in the vicinity of an outermost side of the opening diameter of the condenser lens 1. The reflective mirror 9 is disposed so as to reflect the laser beam introduced from the direction crossing the transmitted illuminative light path T substantially at right angles substantially at right angles. Reference numeral 10 represents a mirror holding section which holds the reflective mirror 9 so as to allow the mirror 9 to be movable in the direction

crossing the transmitted illuminative light path T at substantially at right angles. Reference numeral 11 represents a micrometer head which abuts on the mirror holding section 10, 12 represents a spring which has a tensile force to urge the micrometer head 11 to abut on and be kept by the mirror holding section 10, and 13 represents a micrometer operation section which directly moves the micrometer head 11 by rotation.

[0051]

Here, the translatory direction of the micrometer head 11 agrees with the direction crossing the transmitted illuminative light path T substantially at right angles. Therefore, when the micrometer operation section 13 is rotated, the reflective mirror 9 moves in a translatory manner in a direction crossing the transmitted illuminative light path T substantially at right angles.

[0052]

Reference numeral 14 represents a laser oscillation unit. Reference numeral 15 represents a fiber, preferably a single mode fiber, which introduces a laser beam oscillated by the laser oscillation unit 14. Reference numeral 16 represents a fiber emission end which emits the laser beam as a divergent ray. Reference numeral 17 represents a condensing lens which converts a divergent ray emitted from the fiber emission end 16 into a convergent ray, and condenses the ray in the vicinity of a front focal position of the condenser lens 1 via the reflective mirror 9. The elements denoted in the numerical order, 9 through 17, constitute a laser introduction section L₁. The laser introduction section L₁ is fixed to the

microscope on a base 18.

[0053]

Additionally, a wavelength of the laser beam introduced from the laser introduction section L₁ to the transmitted illuminative light path T, that is, an oscillation wavelength of the laser oscillation unit 14 has a single wavelength λ_{L1} .

An image forming lens 22 is disposed on an observation optical path emitted from the objective lens 6. An absorption filter 24 is disposed on an extending portion of the observation optical path from the image forming lens 22. Further, an image pick-up device 25 is disposed in a focal position of the image forming lens 22 on an extending portion of the observation optical path from the absorption filter 24. The absorption filter 24 is a band pass filter which passes a light only of a specific wavelength band λ_{E1} longer than a wavelength λ_{L1} of a laser beam output.

[0054]

Next, an operation of the TIRFM in this embodiment will be described.

The laser beam having the wavelength λ_{L1} oscillated by the laser oscillation unit 14 is introduced into the fiber 15, and emitted as a divergent ray from the fiber emission end 16. The laser beam emitted as the divergent ray is converted to the convergent ray through the condensing lens 17 and is incident upon the reflective mirror 9. The laser beam incident upon the reflective mirror 9 is reflected on the condenser lens 1 side in the vicinity of the outermost side of the transmitted illuminative light path T. The laser beam reflected by the

reflective mirror 9 is once condensed in the vicinity of the front focal position of the condenser lens 1. Moreover, the laser beam is incident upon the condenser lens 1, and is emitted as a parallel ray advancing in an oblique direction from the condenser lens 1. The laser beam emitted from the condenser lens 1 is transmitted through the immersion oil 2 and is incident upon the boundary surface between the slide glass 3 and the specimen 4.

[0055]

When the incidence angle θ_{L1} of the laser beam upon the boundary surface between the slide glass 3 and the specimen 4 is larger than the critical angle of the total internal reflection, the laser beam is totally reflected by the boundary surface. Accordingly, the evanescent light leaks on a specimen 4 side. The specific fluorescent substance existing in the specimen 4 is excited by the evanescent light having the wavelength λ_{L1} . By this excitation, the fluorescent substance emits the fluorescence such that a maximum luminance wavelength of the fluorescence is in a transmission wavelength band λ_{E1} of the absorption filter 24. The fluorescence is incident upon the objective lens 6 through the cover glass 5. Furthermore, the fluorescence is transmitted through the image forming lens 22 and absorption filter 24, and is incident upon the image pick-up device 25. The image pick-up device 25 picks up a fluorescent image of the wavelength band λ_{E1} .

[0056]

On the other hand, as described above, when the micrometer operation section 13 is rotated, the reflective

mirror 9 moves in the translatory manner in the direction crossing the transmitted illuminative light path T substantially at right angles. When the position of the reflective mirror 9 moves in the direction crossing the transmitted illuminative light path T substantially at right angles, the incidence position of the laser beam upon the condenser lens 1 moves. Accordingly, an emission angle of the laser beam emitted from the condenser lens 1, that is, an incidence angle θ_{L1} of the laser beam upon the boundary surface between the slide glass 3 and specimen 4 changes.

[0057]

As described above, the leak-out depth of the evanescent light in the total internal reflection illumination changes with the incidence angle of the laser beam upon the boundary surface. Therefore, the micrometer operation section 13 is rotated to slightly move the reflective mirror 9 in the direction crossing the transmitted illuminative light path T substantially at right angles. That is, when the reflective mirror 9 is brought close to or far from the optical axis of the transmitted illuminative light path T, the leak-out depth d_{L1} of the evanescent light can be optionally changed. It is to be noted that when the transmission illuminating observation is performed using the illuminative light output from the transmitted illuminative light source 7, the reflective mirror 9 is completely retreated from the transmitted illuminative light path T.

[0058]

As described above, with the constitution and operation

of the TIRFM according to the first embodiment of the present invention, a maximum incidence angle θ_{\max} of the laser beam depends only on the NA of the condenser lens 1 represented by the above equation (5). Therefore, fluorescence observation by the total internal reflection illumination is possible regardless of the NA or the magnification of the objective lens 6.

[0059]

The laser introduction section L_1 is disposed farther than the condenser lens 1 from the specimen 4. Accordingly, a space in the vicinity of the specimen 4 is not compressed. In addition, the laser introduction section L_1 has a simple structure and also has a narrow operation range. Therefore, for the laser introduction section L_1 , the TIRFM itself can be compact, and the TIRFM can have a superior operation property. (Modification) The first embodiment of the present invention described using FIG. 1 above may also be modified as follows. A glass bottom dish may also be used instead of the slide glass 3. Accordingly, the cover glass 5 may also be omitted. In this case, when an operating distance of the objective lens 6 is short, an immersion objective lens is used in the objective lens 6.

[0060]

For example, an electromotive motor, piezo-actuator or the like may also be used instead of the micrometer for moving the reflective mirror 9.

The reflective mirror 9 is fixed. The fiber emission end 16 and condensing lens 17 are integrally moved in a direction

parallel to the transmitted illuminative light path T. Even with this constitution, a function similar to that of the first embodiment is obtained. In this case, the fiber emission end 16 and condensing lens 17 are moved, for example, using a micrometer, electromotive motor, piezo-actuator, or the like.

[0061]

The first embodiment described above is applied to an erected type microscope. However, the first embodiment is applicable also to an inverted microscope.

[0062]

The observation optical path is not limited to the constitution described in this embodiment, and can be optionally constituted as long as the fluorescence wavelength with respect to the excited wavelength of the laser introduction section L₁ is selected and the image can be picked up.

[0063]

In the first embodiment, the laser beam emitted from the fiber emission end 16, which is the divergent ray, is converted to the convergent ray by the single condensing lens 17, and the convergent ray is reflected by the reflective mirror 9 and condensed in the vicinity of the front focal position of the condenser lens 1. The present invention, however, is not limited to this. For example, the laser beam emitted from the fiber emission end 16 is converted to a parallel light flux by a single lens or a plurality of lenses, the condensing lens is disposed in an optional position on the optical path from when the parallel light flux is reflected by the reflective mirror 9

and is incident upon the vicinity of the front focal position of the condenser lens 1, and the light may be condensed by the condensing lens.

(Second embodiment) FIG. 2 is a constitution diagram showing a second embodiment of the present invention. It is to be noted that the same parts as those of FIG. 1 are denoted with the same reference numerals and the detailed description is omitted.

[0064]

In FIG. 2, reference numeral 19 represents a conversion lens unit integrally detachably inserted with respect to a laser introductory optical path between the fiber emission end 16 and the condensing lens 17. FIG. 3 shows partially enlarged plan views of the conversion lens unit 19 shown in FIG. 2 and is used for the explanation of the constitution and arrangement of the conversion lens unit 19 of the second embodiment.

[0065]

In FIG. 3, reference numeral 19a represents a convex lens which converts the NA of a divergent laser beam emitted from the fiber emission end 16. Reference numeral 19b represents a concave lens which adjusts the focal distance so that the NA-converted laser beam is condensed in the vicinity of the front focal position of the condenser lens 1 via the condensing lens 17. Reference numeral 20 represents a knob used to detachably attach the conversion lens unit 19 onto the laser introductory optical path. Here, when the knob 20 is pulled out as shown in FIG. 3(a), the conversion lens unit 19 comes out of the laser introductory optical path. When the knob 20

is pushed in as shown in FIG. 3(b), the conversion lens unit 19 is inserted into the laser introductory optical path.

[0066]

Next, the operation of the TIRFM in this embodiment will be described.

When the knob 20 is pushed inwards, the conversion lens unit 19 is inserted into the laser introductory optical path. The NA of the laser beam diverged from the fiber emission end 16 is converted by the convex lens 19a in this state.

Thereafter, the laser beam is condensed in the vicinity of the front focal position of the condenser lens 1 through the concave lens 19b and condensing lens 17. At this time, the incidence NA of the laser beam upon the condenser lens 1 is reduced as compared with a case where the convex lens 19a and concave lens 19b deviate from the laser introductory optical path. Accordingly, the laser beam is condensed in the vicinity of the front focal position of the condenser lens 1 at a small incidence NA.

[0067]

As a result, a ray flux diameter of the parallel ray advancing in an oblique direction after passing through the condenser lens 1, that is, a laser beam irradiation range in the specimen 4 is condensed. Accordingly, energy density of the laser beam increases.

[0068]

As described above, with the constitution and operation of the TIRFM according to the second embodiment of the present invention, the conversion lens unit 19 is detachably inserted

into the laser introductory optical path. Accordingly, the irradiation range of the laser beam on the specimen 4, and so the energy density of the laser beam can be converted. Therefore, when the weak fluorescence is to be observed with a strong power, the conversion lens unit 19 is inserted into the laser introductory optical path. In the fluorescence observation of the broad range, the conversion lens unit 19 is detached from the laser introductory optical path. The specimen 4 is selectively used in accordance with the application of the observation in this manner.

(Modification) The second embodiment of the present invention described above may also be modified as follows.

Inserting/detaching means of the conversion lens unit 19 is not limited to the knob and may also be, for example, the electromotive motor and can be freely constituted.

[0069]

In the above-described embodiment, as shown in FIG. 3, the conversion lens unit 19 is not limited to a combination of the convex lens 19a and concave lens 19b, in which the convex lens 19a converts the NA of a divergent laser beam emitted from the fiber emission end 16, and the concave lens 19b adjusts the focal distance so that the NA-converted laser beam is condensed in the vicinity of the front focal position of the condenser lens 1, and may also be a combination of any convex and concave lenses, from which the same function is obtained.

[0070]

In the second embodiment described above, for the conversion lens unit 19, one type of unit is constituted to be

detachably inserted between the fiber emission end 16 and condensing lens 17, but the present invention is not limited to this. For example, the conversion lens unit 19 which converts an incidence NA of the laser beam to a different incidence NA may be selectively detachably inserted between the fiber emission end 16 and condensing lens 17. With the constitution, the specimen can be irradiated with the laser beams having three or more different incidence NA. Accordingly, a finer irradiation range of the laser beam, and further the energy density are adjustable.

(Third embodiment) FIGS. 4 and 5 are constitution diagrams showing a third embodiment of the present invention. It is to be noted that the same parts as those of FIGS. 2 and 3 are denoted with the same reference numerals and the detailed description is omitted.

[0071]

In FIG. 4, reference numeral 61 represents an objective lens for high-magnification observation, and 62 represents an objective lens for low-magnification observation. Here, the laser beam irradiation range is set so as to substantially agree with an observation range of the objective lens for high-magnification observation 61 in a state in which the conversion lens unit 19 is inserted into the laser introductory optical path. The laser beam irradiation range is set so as to substantially agree with the observation range of the objective lens for low-magnification observation 62 in a state in which the conversion lens unit 19 is detached from the laser introductory optical path.

[0072]

In FIG. 5, reference numeral 63 represents an observing magnification switching means used for selectively arranging the objective lens for high-magnification observation 61 and objective lens for low-magnification observation 62 on the observation optical path. The observation magnification switching means 63 includes, for example, an electromotive motor and the like.

[0073]

Reference numeral 64 represents an irradiation range switching means used for detachably inserting the conversion lens unit 19 into the laser introductory optical path. The irradiation range switching means includes, for example, an electromotive motor and the like. Further, reference numeral 65 represents a control section (hereinafter referred to as a PC) which transmits a driving signal of the observing magnification switching means 63 and irradiation range switching means 64. Reference numeral 66 represents a driver which receives the driving signal sent from the PC 65 to drive the observing magnification switching means 63 and irradiation range switching means 64.

[0074]

As described above, with the constitution of the TIRFM according to the third embodiment of the present invention, when the objective lens for high-magnification observation 61 is disposed on the observation optical path, the conversion lens unit 19 is inserted into the laser introductory optical path. On the other hand, when the objective lens for

low-magnification observation 62 is inserted into the observation optical path, the conversion lens unit 19 deviates from the laser introductory optical path. Accordingly, when the observation magnification is switched, the laser beam irradiation range can also be switched in cooperation so as to substantially agree with a size of visual field for observation.

(Modification) The third embodiment of the present invention described above may also be modified as follows.

Three or more objective lenses may also be used. In this case, a plurality of conversion lens units having different reduction ratios of the respective irradiation range are disposed corresponding to the plurality of objective lenses. The PC 65 inserts the corresponding conversion lens unit into the laser introductory optical path in cooperation with the switching of each objective lens.

[0075]

When the cooperative operation is not required, the observing magnification switching means 63 and irradiation range switching means 64 may be independently switched.

In this case, the switching of the respective observing magnification switching means 63 and irradiation range switching means 64 may also be performed manually.

(Fourth embodiment) FIG. 6 is a constitution diagram showing a fourth embodiment of the present invention. It is to be noted that the same parts as those described so far are denoted with the same reference numerals and the detailed description is omitted.

[0076]

In FIG. 6, reference numeral 191 represents a convex lens which is disposed movably in the optical axis direction of the laser introductory optical path between the fiber emission end 16 and the condensing lens 17, and converts the NA of the divergent laser beam emitted from the fiber emission end 16. Reference numeral 192 represents a concave lens which is disposed movably in the optical axis direction of the laser introductory optical path between the convex lens 191 and the condensing lens 17, and adjusts the focal distance to condense the laser beam whose NA is converted by the convex lens 191 in the vicinity of the front focal position of the condenser lens 1 via the condensing lens 17. Reference numerals 193 and 194 are moving means used for moving the convex lens 191 and concave lens 192, respectively, in the optical axis direction of the laser introductory optical path. The moving means 193 and 194 include, for example, an electromotive motor and the like.

[0077]

Reference numeral 195 represents a PC which transmits a driving signal of the moving means 193 and 194. Reference numeral 196 represents a driver which receives the driving signal sent from the PC 195 to drive the respective moving means 193 and 194. The PC 195 executes driving program to determine a moving position of the concave lens 192 for adjusting the condensing position of the laser beam in the vicinity of the front focal position of the condenser lens 1 in accordance with the positional movement of the convex lens 191,

and controls a moving/driving amount of the respective moving means 193 and 194 based on the information of the determined moving position of the concave lens 192.

[0078]

Next, the operation of the TIRFM in this embodiment will be described.

The moving means 193 and 194 are driven by the driver 196 based on the driving signal of the PC 195. The moving means 193 and 194 move the convex lens 191 and concave lens 192, respectively, in the optical axis direction of the laser introductory optical path in such manner as illustrated in FIGS. 6(a) through 6(b). At this time, the PC 195 executes driving program to determine a moving position of the concave lens 192 for adjusting the condensing position of the laser beam in the vicinity of the front focal position of the condenser lens 1 in accordance with the positional movement of the convex lens 191, and controls a moving/driving amount of the respective moving means 193 and 194 based on the information of the determined moving position of the concave lens 192.

[0079]

Accordingly, the NA of the divergent laser beam emitted from the fiber emission end 16 is converted by the convex lens 191 whose position is moved. The focal distance of the laser beam whose NA has been converted is adjusted by passage through the concave lens 192 and condensing lens 17 which have been moved in accordance with the movement of the convex lens 191. As a result, the laser beam is condensed in the vicinity of the

front focal position of the condenser lens 1.

[0080]

Therefore, only the incidence NA can be continuously converted without changing the condensing position of the laser beam. As a result, the ray flux diameter of the parallel ray advancing in the oblique direction after passing through the condenser lens 1, that is, the size of the laser beam irradiation range in the specimen 4 is continuously converted. The energy density of the laser beam is also continuously converted in accordance with the change of the laser beam irradiation range.

[0081]

As described above, with the constitution of the TIRFM according to the fourth embodiment of the present invention, the laser beam irradiation range in the specimen 4, that is, the energy density of the laser beam can be continuously converted. When the weak fluorescence is to be detected with a strong power, the laser beam irradiation range is condensed. For the fluorescence observation in the broad range, the laser beam irradiation range is enlarged. In this manner, it is possible to selectively use the specimen 4 in accordance with the applications such as the observation purpose.

(Modification) The fourth embodiment of the present invention described above may also be modified as follows.

[0082]

FIG. 7 is a constitution diagram of the modification of this embodiment. It is to be noted that the same parts as those of FIG. 6 are denoted with the same reference numerals,

and the detailed description is omitted.

In FIG. 7, reference numeral 610 represents an objective lens revolver which holds a plurality of objective lenses having different observation magnifications (611 to 616 in FIG. 7), and selectively dispose one of the objective lenses 611 to 616 on the observation optical path. Reference numeral 617 represents an observation magnification switching means used for switching the objective lenses by rotation of the revolver 610. The observation magnification switching means 617 includes, for example, an electromotive motor and the like.

[0083]

The driver 196 drives the observation magnification switching means 617 based on a driving signal of the PC 195. The PC 195 executes the driving program to select the observation magnification of the objective lenses and drive the switching means 617. Moreover, the PC 195 determines the respective positions of the convex lens 191 and concave lens 192 so as to obtain the laser beam irradiation range which substantially agrees with the observation range in the observation magnifications of the objective lenses 611 to 616, and drives the moving means 193 and 194 based on the determined positional information.

[0084]

With this constitution, the laser beam irradiation range substantially agreeing with the observation range of the selected objective lens can be freely set and switched in cooperation with the optional observation magnification of the objective lens.

[0085]

In the above embodiment, the zoom lens unit shown in FIG. 6 is constituted by a combination of the convex lens 191 which converts the NA of a divergent laser beam emitted from the fiber emission end 16, and the concave lens 192 which is movably disposed in the optical axis direction of the laser introductory optical path between the convex lens 191 and condensing lens 17 and adjusts the focal distance so that the laser beam NA-converted by the convex lens 191 is condensed in the vicinity of the front focal position of the condenser lens 1 via the condensing lens 17, that is, the combination of a convex lens and a concave lens. The zoom lens unit may also be a combination of a convex and a concave lens, from which the same function is obtained.

(Fifth embodiment) FIG. 8 is a constitution diagram showing a fifth embodiment of the present invention. It is to be noted that the same parts as those of FIG. 1 are denoted with the same reference numerals and the detailed description is omitted.

[0086]

In FIG. 8, reference symbol L₂ represents a laser introduction section which is disposed facing the laser introduction section L₁ via the transmitted illuminative light path T, and has a constitution identical with the laser introduction section L₁. The laser introduction section L₂ is fixed to the microscope on the base 18 in the same manner as the laser introduction section L₁.

[0087]

Here, assuming that the laser beam introduced into the transmitted illuminative light path T from the laser introduction section L₂ has a single wavelength λ_{L2} .

FIG. 9 shows a bottom view of the TIRFM shown in FIG. 8 and is used for the explanation about the arrangement of the laser introduction sections in the fifth embodiment.

[0088]

In FIG. 9, L₃ and L₄ are laser introduction sections which are omitted in FIG. 8. The laser introduction sections L₃ and L₄ are also fixed to the microscope on the base 18 in the same manner as the laser introduction sections L₁ and L₂.

[0089]

A plurality of the respective laser introduction sections are radially disposed in the direction crossing the optical axis of the transmitted illuminative light path T substantially at right angles, centering on the transmitted illuminative light path T.

Here, assuming that the laser beams introduced into the transmitted illuminative light path T from the laser introduction sections L₁ and L₄ have single wavelengths λ_{L3} and λ_{L4} , respectively.

[0090]

Next, the constitution on an observation optical path side in this embodiment will be described.

In FIG. 8, reference numeral 21 represents a first dichroic mirror which is disposed on the observation optical path emitted from the objective lens 6. The first dichroic

mirror 21 has characteristics that the mirror reflects the light having a wavelength shorter than a standard wavelength λ_1 and transmits the light having a wavelength longer than the wavelength λ_1 . The first image forming lens 22 is disposed on a reflective optical path of the first dichroic mirror 21, and a second dichroic mirror 23 is disposed on an extending portion of the reflective optical path from the image forming lens 22. The second dichroic mirror 23 has a wavelength λ_2 which is a standard wavelength shorter than the wavelength λ_1 , and has characteristics that the mirror reflects the light on the side of the wavelength shorter than a wavelength λ_2 and transmits the light on the long wavelength side.

[0091]

The first absorption filter 24 is disposed on the reflective optical path of the second dichroic mirror 23. The first image pick-up device 25 is disposed in the focal position of the first image forming lens 22 on an extending portion of the reflective optical path from the first absorption filter 24.

[0092]

The first absorption filter 24 is a band pass filter which transmits only the light having a specific wavelength band λ_{E1} shorter than the wavelength λ_2 . A second absorption filter 26 is disposed on the transmission optical path of the second dichroic mirror 23. A second image pick-up device 27 is disposed in the focal position of the first image forming lens 22 on an extending portion of the transmission optical path from the second absorption filter 26. The second absorption

filter 26 is a band pass filter which transmits the light only of a specific wavelength band λ_{E2} between the wavelengths λ_2 and λ_1 .

[0093]

On the other hand, a second image forming lens 28 is disposed on the transmission optical path of the first dichroic mirror 21. A total internal reflection prism 29 is disposed on an extending portion of the transmission optical path from the second image forming lens 28. A third dichroic mirror 30 is disposed on the reflective optical path of the total internal reflection prism 29.

[0094]

The third dichroic mirror 30 has a wavelength λ_3 which is a standard wavelength longer than the wavelength λ_1 , and has characteristics that the mirror reflects the light on the side of the wavelength shorter than the wavelength λ_3 and transmits the light on the long wavelength side. A third absorption filter 31 is disposed on the reflective optical path of the third dichroic mirror 30. A third image pick-up device 32 is disposed in the focal position of the second image forming lens 28 on an extending portion of the reflective optical path from the third absorption filter 31.

[0095]

The third absorption filter 31 is a band pass filter which transmits the light only of a specific wavelength band λ_{E3} between the wavelengths λ_1 and λ_3 . A fourth absorption filter 33 is disposed on the transmission optical path of the third dichroic mirror 30. A fourth image pick-up device 34 is

disposed in the focal position of the second image forming lens 28 on an extending portion of the transmission optical path from the fourth absorption filter 33.

[0096]

The fourth absorption filter 33 is a band pass filter which transmits the light only of a specific wavelength band λ_{E4} longer than the wavelength λ_3 .

FIG. 10 is a diagram showing a relation among the wavelengths separated by the respective dichroic mirrors and absorption filters described above.

[0097]

Next, the operation of the TIRFM in this embodiment will be described.

The laser beam having the wavelength λ_{L1} oscillated by the laser oscillation unit 14 is introduced into the fiber 15, and is emitted as a divergent ray from the fiber emission end 16. The laser beam emitted as the divergent ray is converted to the convergent ray through the condensing lens 17, and is incident upon the reflective mirror 9. The laser beam incident upon the reflective mirror 9 is reflected on the condenser lens 1 side in the vicinity of the outermost side of the transmitted illuminative light path T. The laser beam reflected by the reflective mirror 9 is once condensed in the vicinity of the front focal position of the condenser lens 1. Moreover, the laser beam is incident upon the condenser lens 1, and is emitted as the parallel ray advancing in the oblique direction from the condenser lens 1. The laser beam emitted from the condenser lens 1 is transmitted through the immersion oil 2 and

is incident upon the boundary surface between the slide glass 3 and specimen 4.

[0098]

When the incidence angle θ_{L1} of the laser beam upon the boundary surface between the slide glass 3 and specimen 4 is larger than the critical angle of the total internal reflection, the laser beam is totally reflected by the boundary surface. Accordingly, the evanescent light leaks on the specimen 4 side.

[0099]

The specific fluorescent substance existing in the specimen 4 is excited by the evanescent light having the wavelength λ_{L1} . By the excitation, the fluorescent substance emits the fluorescence such that the maximum luminance wavelength of the fluorescence is in the transmission wavelength band λ_{E1} of the first absorption filter 24. The fluorescence is incident upon the objective lens 6 through the cover glass 5. Furthermore, the fluorescence is reflected by the dichroic mirror and transmitted through the absorption filter on the observation optical path, and is finally incident upon the first image pick-up device 25. The first image pick-up device 25 picks up the fluorescent image of the wavelength band λ_{E1} .

[0100]

On the other hand, as has been described, when the micrometer operation section 13 is rotated, the reflective mirror 9 moves in the translatory manner in the direction crossing the transmitted illuminative light path T

substantially at right angles. When the position of the reflective mirror 9 moves, the incidence position of the laser beam upon the condenser lens 1 after the laser beam is reflected moves. Accordingly, the emission angle of the laser beam emitted from the condenser lens 1, that is, the incidence angle θ_{L1} of the laser beam upon the boundary surface between the slide glass 3 and specimen 4 changes.

[0101]

As has been described, the leak-out depth of the evanescent light in the total internal reflection illumination is changed according to the incidence angle of the laser beam upon the boundary surface. Therefore, by rotating the micrometer operation section 13 to slightly move the reflective mirror 9, the leak-out depth d_{L1} of the evanescent light can be optionally changed.

[0102]

It is to be noted when the transmission illuminating observation is performed using the illuminative light output from the transmitted illuminative light source 7, the reflective mirror 9 is completely retreated from the transmitted illuminative light path T.

The laser introduction section L₂ is similar to the laser introduction section L₁. When an incidence angle θ_{L2} of the laser beam having a wavelength λ_{L2} incident upon the boundary surface between the slide glass 3 and specimen 4 is changed. Accordingly, a leak-out depth d_{L2} of the evanescent light can be optionally changed.

[0103]

The specific fluorescent substance existing in the specimen 4 is excited by the evanescent light having the wavelength λ_{L2} . By the excitation, the fluorescent substance emits the fluorescence such that the maximum luminance wavelength of the fluorescence is in the transmission wavelength band λ_{E2} of the second absorption filter 26. The fluorescence is incident upon the objective lens 6 through the cover glass 5. Furthermore, the fluorescence is reflected by the dichroic mirror and transmitted through the absorption filter on the observation optical path, and is finally incident upon the second image pick-up device 27. The second image pick-up device 27 picks up the fluorescent image of the wavelength band λ_{E2} .

[0104]

The same is true for the laser introduction sections L₃ and L₄. The specific fluorescent substances excited by the evanescent light having the wavelengths λ_{L3} and λ_{L4} are reflected by the dichroic mirror and transmitted through the absorption filter on each observation optical path, respectively, and are finally incident upon the third image pick-up device 32 and fourth image pick-up device 34, respectively. The third image pick-up device 32 picks up the fluorescent image of the wavelength band λ_{L3} and the fourth image pick-up device 34 picks up the fluorescent image of the wavelength band λ_{L4} .

[0105]

As described above, with the constitution and operation

of the TIRFM according to the fifth embodiment of the present invention, each of the laser introduction sections is disposed farther than the condenser lens 1 from the specimen 4. Accordingly, a space in the vicinity of the specimen 4 is not compressed. In addition, each laser introduction section has a simple structure and also has a narrow operation range. Therefore, for the laser introduction sections, the TIRFM itself can be compact, and a plurality of laser introduction sections can be radially arranged. Moreover, for the laser introduction sections L₁, L₂, L₃, L₄, the respective wavelengths and the leak-out depths of the evanescent light can be individually set.

[0106]

Therefore, each portion in the specimen 4 which is an observation object is sometimes labeled using a plurality of types of fluorescent substances such as CFP, GFP, YFP, RFP. In this case, it is possible to adjust the leak-out depth of the evanescent light having a wavelength for excitation to be optimum in accordance with the depth in which each portion to be labeled is positioned. The respective laser introduction sections are constituted completely independently of one another. Accordingly, optical characteristics can be independently set in accordance with user's application. It is possible to dispose, add, or remove the necessary number of laser introduction sections. An illuminating side (condenser lens side) is separated from an observation side (objective lens side). Accordingly, the dichroic mirror of the multi-band is not required, and the handling is facilitated.

[0107]

As described above, a total internal reflection fluorescence microscope having the following superior system property can be provided. The microscope is compact and is easily handled. A plurality of laser introduction sections can easily be added. The leak-out depths of the evanescent light can be independently set and it is possible to simultaneously irradiate the specimen 4 with the laser beam having a plurality of wavelengths.

(Modification) The fifth embodiment of the present invention described above may also be modified as follows.

The laser introduction sections can further be added to an extra space if any.

[0108]

A glass bottom dish may also be used instead of the slide glass 3. Accordingly, the cover glass 5 may be omitted. In this case, when the operating distance of the objective lens 6 is short, an immersion objective lens is used.

[0109]

The reflective mirror 9 may also be moved using, for example, an electromotive motor, piezo-actuator or the like in addition to the micrometer.

The reflective mirror 9 is fixed. The fiber emission end 16 and condensing lens 17 may be integrally moved in the direction parallel to the optical axis of the transmitted illuminative light path T. With this constitution, the functions similar to those in this embodiment can be obtained. In this case also, the fiber emission end 16 and condensing

lens 17 are movable, for example, using a micrometer, electromotive motor, piezo-actuator, or the like.

[0110]

The embodiment is applicable not only to the erected type microscope but also to the inverted microscope.

[0111]

The constitution on the observation optical path may also be optional as long as the fluorescence wavelength with respect to each excitation wavelength is selected to pick up the image.

[0112]

As shown in FIG. 11, shutters may also be disposed in the respective laser introduction sections to select the introducing and blocking of the laser beam. A shutter controller individually controls the opening/closing of each of the shutters. Accordingly, the laser beam to be introduced into the specimen can be selected.

[0113]

The wavelength of each laser beam introduced from a plurality of laser introduction sections, for example, at least two laser introduction sections is set to be equal. Moreover, the respective laser beams are alternately introduced into the specimen 4 by the opening/closing of the respective shutters while the leak-out depth of each laser beam is changed. As a result, when the observed images by the difference in the leak-out depth of the fluorescence by the same fluorescent substance are compared, it is possible to specify the depth of a generation source of the fluorescence.

[0114]

In the embodiment described above, the laser oscillation units which oscillate the laser beams having a single wavelength are provided in the respective laser introduction sections. The present invention is not limited to this, and the laser oscillation unit may also oscillate a laser beam having a multi-wavelength. For example, a laser combiner shown in FIG. 12 may also be used.

[0115]

The laser combiner shown in FIG. 12 includes laser oscillation units 70a to 70c which oscillate the respective laser beams having different wavelengths, and combines the laser beams together, which are then condensed on the fiber 75. The laser beam emitted from the laser oscillation unit 70c is reflected by the mirror 71. The respective laser beams emitted from the laser oscillation units 70a and 70b are synthesized with the laser beam emitted from the laser oscillation unit 70c by the dichroic mirrors 73 and 72, respectively. The synthesized laser beams are condensed on the fiber 75 by the condensing lens 74. As a result, the laser beam having a multi-wavelength can be oscillated.

[0116]

A plurality of fluorescent substances having different excitation wavelength regions in one laser introduction section can be excited using the laser beam having the multi-wavelength.

(Sixth embodiment) FIG. 13 is a constitution diagram showing a sixth embodiment of the present invention. It is to be noted

that the same parts as those of FIGS. 8 and 9 are denoted with the same reference numerals and the detailed description is omitted.

[0117]

In FIG. 13, reference symbol C₁ represents an optical path length adjustment section disposed on the optical path between the second dichroic mirror 23 and the second image pick-up device 27. The optical path length adjustment section C₁ includes a fixed prism group 41, movable prism 42 provided adjacent to the fixed prism group 41, and moving means 43 which linearly drives the movable prism 42.

[0118]

The movable prism 42 is configured to move in directions in which the optical path length changes from a reference position in the state where the second image pick-up device 27 is located at the image forming position of the first image forming lens 22. The movable means 43 uses, for example, an electromotive motor, piezo-actuator or the like in order to move the movable prism 42 in the translatory manner. Reference numeral 44 is a PC which process images picked up by the respective image pick-up devices, and transmits a driving signal of the moving means 43. Reference numeral 45 is a driver which receives the driving signal from the PC 44 to drive the moving means 43.

[0119]

FIG. 14 is an enlarged view of the inside of the specimen 4 and the observation optical path in this embodiment. In FIG. 14, reference symbol F₁ is a first fluorescent substance

excited by the wavelength λ_{L1} of the laser beam introduced from the laser introduction section L₁. The maximum luminance wavelength of the fluorescence is in the transmission wavelength band λ_{E1} of the first absorption filter 24. Reference symbol F₂ is a second fluorescent substance excited by the wavelength λ_{L2} of the laser beam introduced from the laser introduction section L₂. The maximum luminance wavelength of the fluorescence is in the transmission wavelength band λ_{E2} of the second absorption filter 26.

[0120]

The respective fluorescent substances are introduced into the cell in the specimen 4 so as to be peculiarly developed in specific small organs. The first fluorescent substance F₁ is developed in the small organ in the vicinity of the cell film. The second fluorescent substance F₂ is developed in the small organ positioned slightly distant from the cell film.

[0121]

The leak-out depth d_{L1} of the evanescent light for exciting the fluorescent substance F₁ is set to be shallow. On the other hand, the leak-out depth d_{L2} of the evanescent light for exciting the fluorescent substance F₂ is set to be larger than the leak-out depth d_{L1}. A focal point of the objective lens 6 is adjusted to the small organ in which the first fluorescent substance F₁ is developed.

[0122]

Therefore, the position of the small organ in which the second fluorescent substance F₂ is developed is in the vicinity of the objective lens 6 side rather than the focal position of

the objective lens 6.

Next, the operation of the TIRFM in this embodiment will be described.

[0123]

When the micrometer rotation section 13 of the laser introduction section L_1 is rotated, the depth reaching the first fluorescent substance F_1 of the evanescent light having the wavelength λ_{L1} is adjusted. Similarly, the depth reaching the second fluorescent substance F_2 of the evanescent light having the wavelength λ_{L2} from the laser introduction section L_2 is adjusted.

[0124]

The fluorescence emitted from the first fluorescent substance F_1 in the focal position of the objective lens 6 turns to a parallel ray through the objective lens 6, is reflected by the first dichroic mirror 21, forms a convergent ray through the first image forming lens 22, is passed through the second dichroic mirror 23 and first absorption filter 24, and is formed into an image on the image pick-up surface of the first image pick-up device 25.

[0125]

On the other hand, the fluorescence emitted from the second fluorescent substance F_2 in a position deviating on the objective lens 6 side from the focal position of the objective lens 6 is passed through the objective lens 6 to form a slightly divergent ray as shown by a dotted line in FIG. 14. The fluorescence is reflected by the first dichroic mirror 21 and passed through the first image forming lens 22 to form a

convergent ray. At this time, the image forming position after the second dichroic mirror 23, optical path length adjustment section C₁, and second absorption filter 26 corresponds to the position after the image pick-up surface of the second image pick-up device 27 in a state in which the movable prism 42 is in a standard position. Accordingly, the fluorescence image of the second fluorescent substance F₂ picked up by the second image pick-up device 27 forms an image which is out of focus.

[0126]

The PC 44 emits a driving signal to the driver 45. Accordingly, the moving means 43 moves the movable prism 42 in a direction distant from the fixed prism group 41 in the translatory manner. By the translatory movement, the optical path length is extended reaching the second image pick-up device 27. The image forming position of the fluorescence emitted from the second fluorescent substance F₂ is close to the image pick-up surface of the second image pick-up device 27.

[0127]

FIG. 15 shows a state in which the image forming position of the fluorescence emitted from the second fluorescent substance F₂ agrees with the image pick-up surface of the second image pick-up device 27. The fluorescence emitted from the second fluorescent substance F₂ in the position deviating on the objective lens 6 side from the focal position of the objective lens 6 is formed into an image on the image pick-up surface of the second image pick-up device 27.

[0128]

At this time, the PC 44 calculates a positional deviation of the second fluorescent substance F_2 with respect to the focal position of the objective lens 6 based on the movement from the reference position of the movable prism 42. Moreover, the PC 44 calculates a deviation of enlargement magnification on the image pick-up surface of the second image pick-up device 27. Moreover, the PC 44 performs an image processing to correct the magnification of the picked-up image.

[0129]

As described above, with the constitution and operation of the TIRFM according to the sixth embodiment of the present invention, the objective lens 6 is fixed, and the surfaces having the different depths on the specimen 4 are subjected to total internal reflection illumination, and can be simultaneously observed with high contrast. For example, small organs in the different depth positions on the specimen 4 such as the small organ in which the first fluorescent substance F_1 is developed and the small organ in which the second fluorescent substance F_2 is developed are subjected to the total internal reflection illumination, and can be simultaneously observed with the high contrast.

(Modification) The sixth embodiment may also be modified as follows.

An optical path length adjustment section constituted in the same manner as in the optical path length adjustment section C_1 may also be disposed on each of divided observation optical paths extending to image pick-up devices other than the

second image pick-up device 27.

[0130]

In this case, the driver 45 includes a plurality of channels for driving a plurality of optical path length adjustment sections. The respective channels are independently controlled by a driving signal from the PC 44.

Further, a similar effect is obtained, for example, even when the second image pick-up device 27 itself is linearly moved in a translatory manner in a direction parallel to the optical path instead of disposing the optical path length adjustment section C₁.

[0131]

The similar effect is obtained, for example, even when the other image pick-up devices are driven similarly.

Also in this embodiment, in the same manner as in the fifth embodiment, the shutters which select the introducing and blocking of the laser beam may also be disposed in each of the laser introduction sections, as shown in FIG. 11. When the opening/closing of the respective shutters is controlled by the shutter controller, the laser beam to be introduced into the specimen can be selected.

[0132]

The wavelengths of the laser beams introduced from a plurality of laser introduction sections, for example, at least two laser introduction sections are set to be equal. Moreover, the respective laser beams are alternately introduced into the specimen by the opening/closing the shutters while the leak-out depth of the fluorescence is changed. As a result, when the

observed images by the difference in the leak-out depth of the fluorescence by the same fluorescent substance are compared, it is possible to specify the depth of the generation source of the fluorescence.

[0133]

[Advantages of the Invention]

According to the present invention, it is possible to provide a total internal reflection fluorescence microscope which enables an object lens having a magnification of lower than 60 times to achieve total internal reflection fluorescence observation, and which is compact and does not compress the operation space of a specimen with a superior operation property.

[0134]

Further, it is possible to provide a total internal reflection fluorescence microscope which can convert the laser beam irradiation range in accordance with the observation range of an objective lens having different magnifications.

Further, it is possible to provide a total internal reflection fluorescence microscope in which a plurality of laser beam introduction sections are provided, allowing evanescent lights having different wavelengths and leak-out depths to be simultaneously irradiated.

[0135]

Moreover, it is an object of the invention to provide a total internal reflection fluorescence microscope in which a combination of evanescent lights having different depths is used for illumination, which allows surfaces having different

depths on the specimen to be simultaneously observed with a high contrast.

[Brief Description of the Drawings]

[FIG. 1]

A constitution diagram showing a first embodiment of the present invention.

[FIG. 2]

A constitution diagram showing a second embodiment of the present invention.

[FIG. 3]

FIGS. 3(a) and 3(b) are enlarged plan views of a conversion lens unit according to the second embodiment of the present invention.

[FIG. 4]

A constitution diagram showing a third embodiment of the present invention.

[FIG. 5]

A partial constitution diagram showing the third embodiment of the present invention.

[FIG. 6]

FIGS. 6(a) and 6(b) are partial constitution diagrams showing a fourth embodiment of the present invention.

[FIG. 7]

A constitution diagram showing a modification of the fourth embodiment of the present invention.

[FIG. 8]

A constitution diagram showing a fifth embodiment of the present invention.

[FIG. 9]

A partial constitution diagram showing a fifth embodiment of the present invention.

[FIG. 10]

A diagram showing a relation between wavelengths separated by each dichroic mirror and absorption filter in the fifth embodiment of the present invention.

[FIG. 11]

A partial constitution diagram showing a modification of the fifth embodiment of the present invention.

[FIG. 12]

A partial constitution diagram showing a modification of the fifth embodiment of the present invention.

[FIG. 13]

A constitution diagram showing a sixth embodiment of the present invention.

[FIG. 14]

A partial constitution diagram showing the sixth embodiment of the present invention.

[FIG. 15]

A partial constitution diagram showing the sixth embodiment of the present invention.

[FIG. 16]

FIGS. 16(a), 16(b) and 16(c) are diagrams each showing a function of a conventional TIRFM.

[FIG. 17]

A constitution diagram of the convention TIRFM.

[FIG. 18]

A constitution diagram of the convention TIRFM.

[Explanation of Reference Symbols]

- 1: Condenser lens
- 2: Immersion oil
- 3: Slide glass
- 4: Specimen
- 5: Cover glass
- 6: Objective lens
- 7: Transmitted illuminative light source
- 8: Collector lens
- 9: Reflective mirror
- 10: Mirror holding section
- 11: Micrometer head
- 12: Spring
- 13: Micrometer operation section
- 14: Laser oscillation unit
- 15: Fiber
- 16: Fiber emission end
- 17: Condensing lens
- 18: Base
- 19: Conversion lens unit
- 19a: Convex lens
- 19b: Concave lens
- 20: Knob
- 21: First dichroic mirror
- 22: Image forming lens
- 23: Second dichroic mirror

24: (First) absorption filter
25: (First) image pick-up device
26: Second emission filter
27: Second image pick-up device
28: Image forming lens
29: Total internal reflection prism
30: Third dichroic mirror
31: Third absorption filter
32: Third image pick-up device
33: Fourth absorption filter
34: Fourth pick-up image device
41: Fixed prism group
42: Movable prism
43: Moving means
44: PC
45: Driver
61: Objective lens for high-magnification observation
62: Objective lens for low-magnification observation
63: Observing magnification switching means
64: Irradiation range switching means
65: PC
66: Driver
70a to 70c: Laser oscillation units
71: Reflective mirror
72, 73: Dichroic mirror
74: Condensing lens
75: Fiber
101: Specimen

102: Cover glass

103: Immersion oil

104: Objective lens

106: Laser beam

107: Mirror

108a: Mirror

108b: Mirror

150: Laser illuminating device

160: Microscope

191: Convex lens

192: Concave lens

193: Moving means

194: Moving means

195: PC

196: Driver

261: Point ball lens

261a: Side surface

610 to 616: Objective lenses

617: Observing magnification switching means

L₁ to L₄: Laser introduction sections

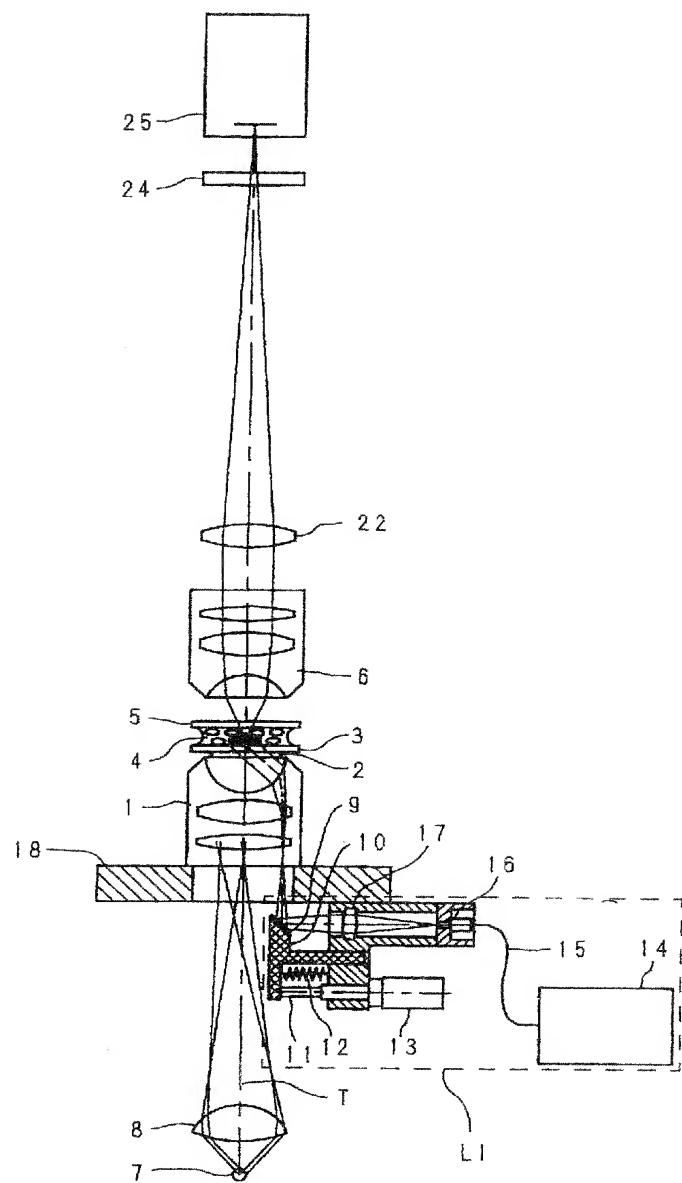
C₁: Optical path length adjustment section

NAME OF DOCUMENT

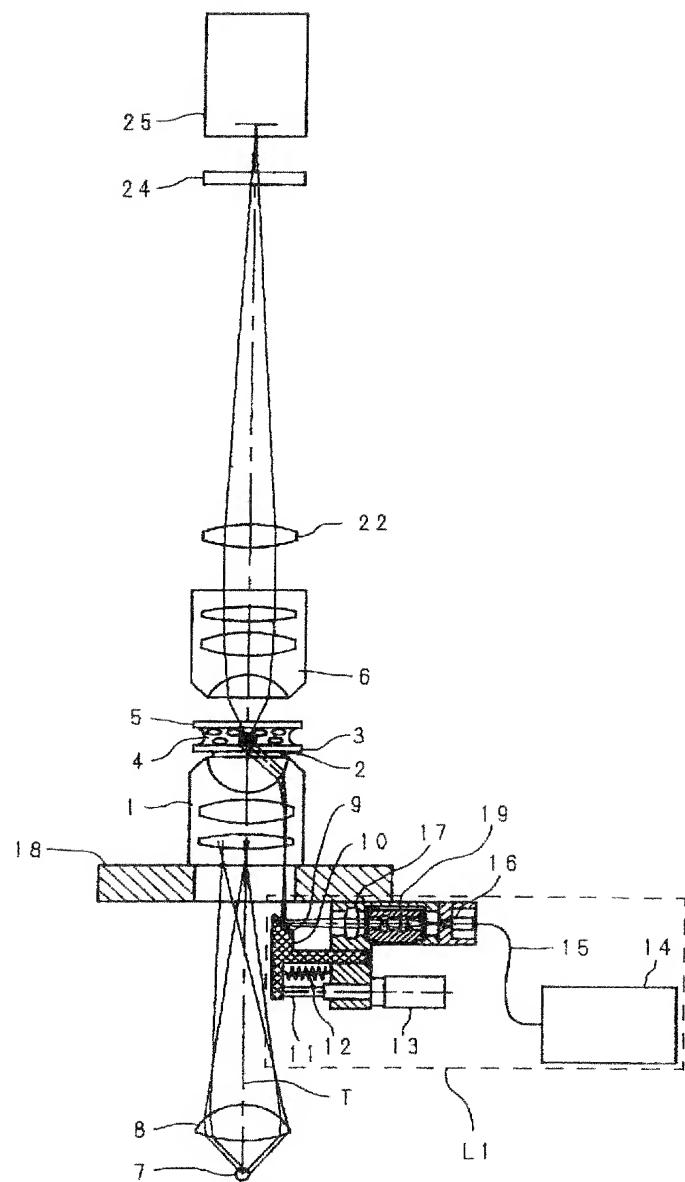
【書類名】 図面

DRAWINGS

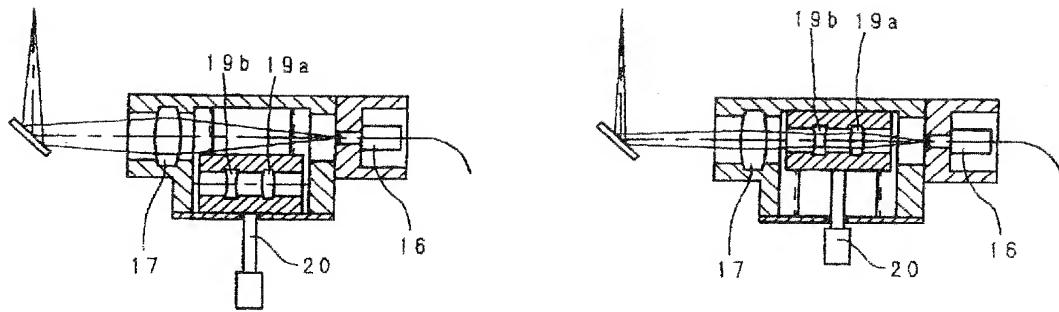
【図 1】 FIG. 1



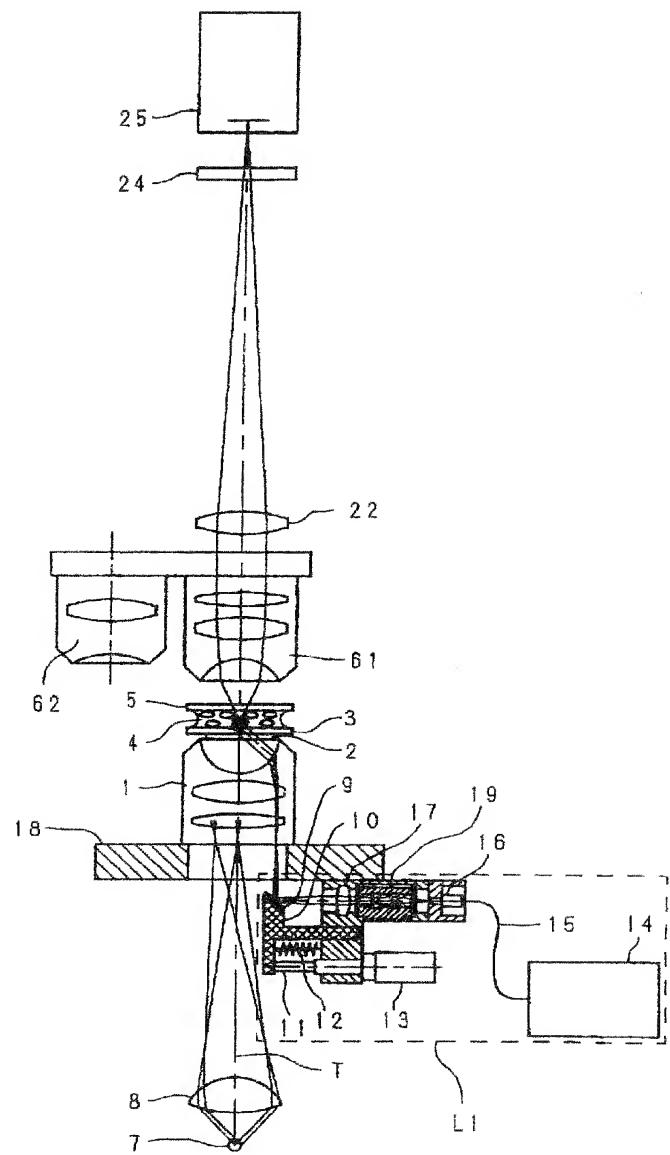
【図2】 FIG. 2



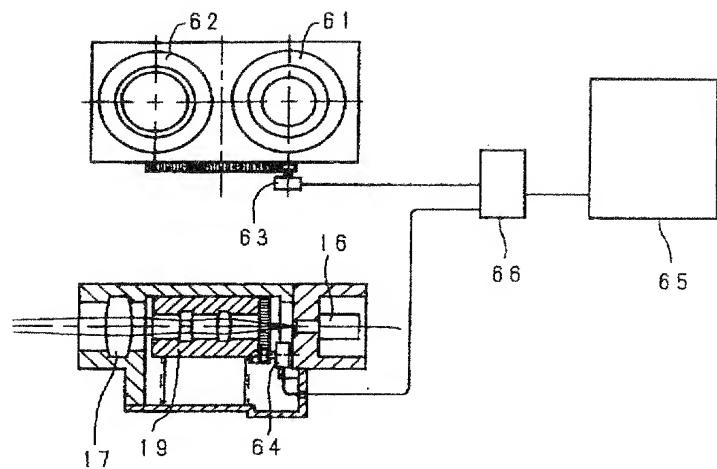
【図3】 FIG. 3



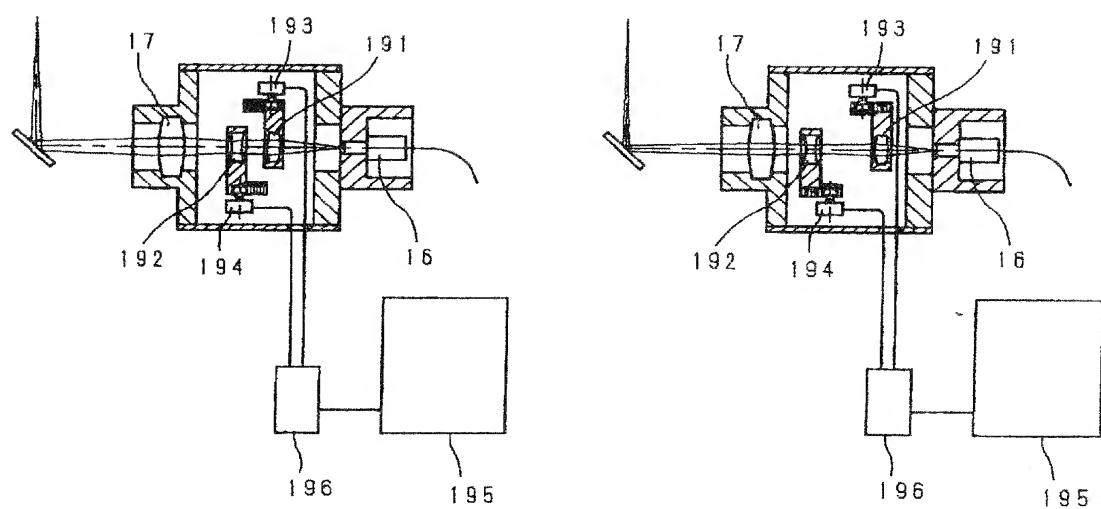
【図 4】 FIG. 4



【図 5】 FIG. 5

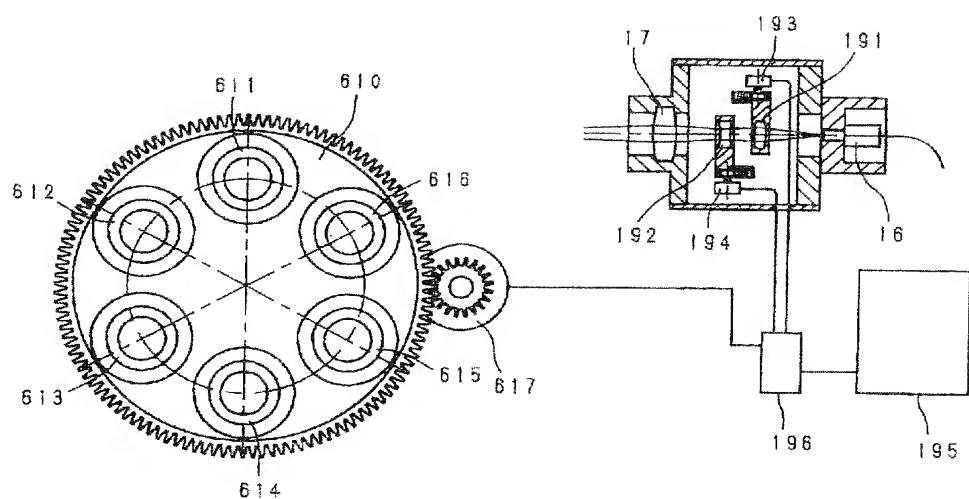


【図 6】 FIG. 6



【図 7】

FIG. 7



【図8】 FIG. 8

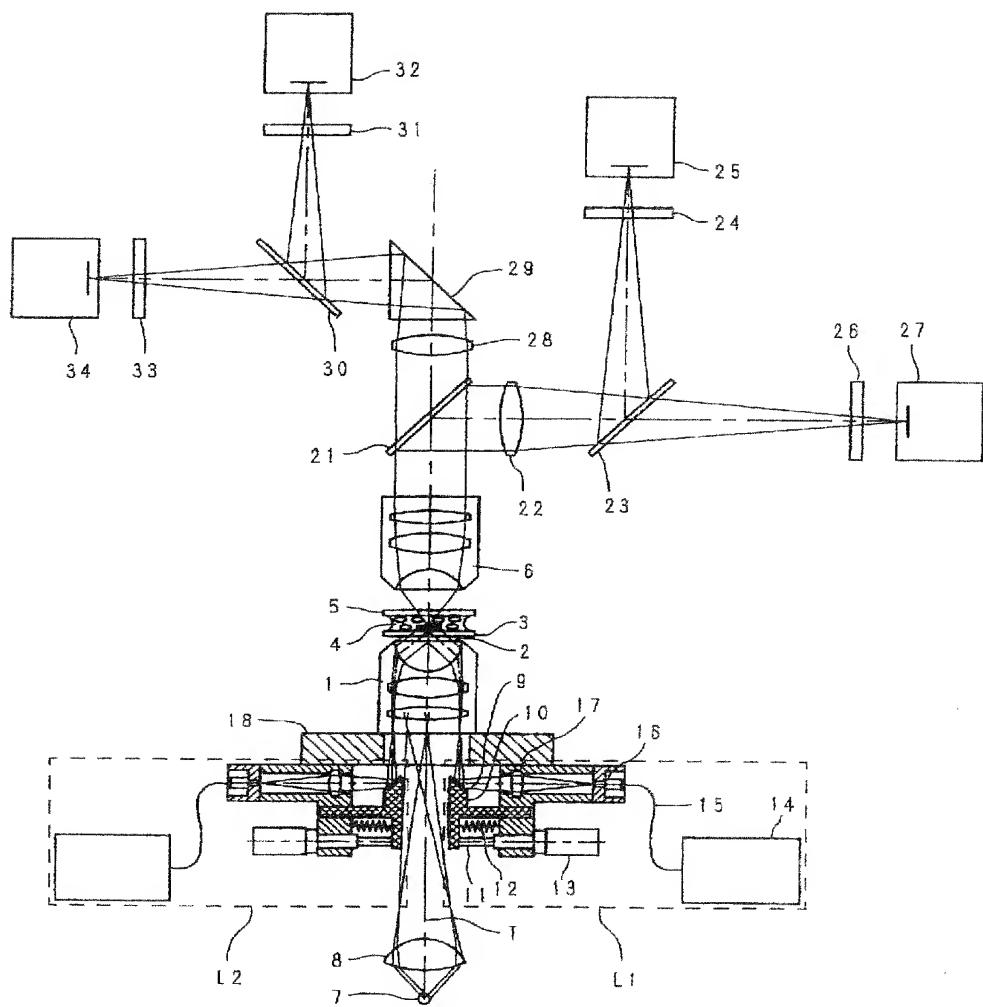


FIG. 9

【図9】

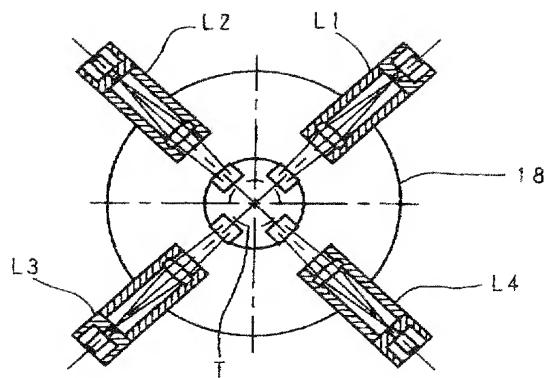


FIG. 10

【図10】

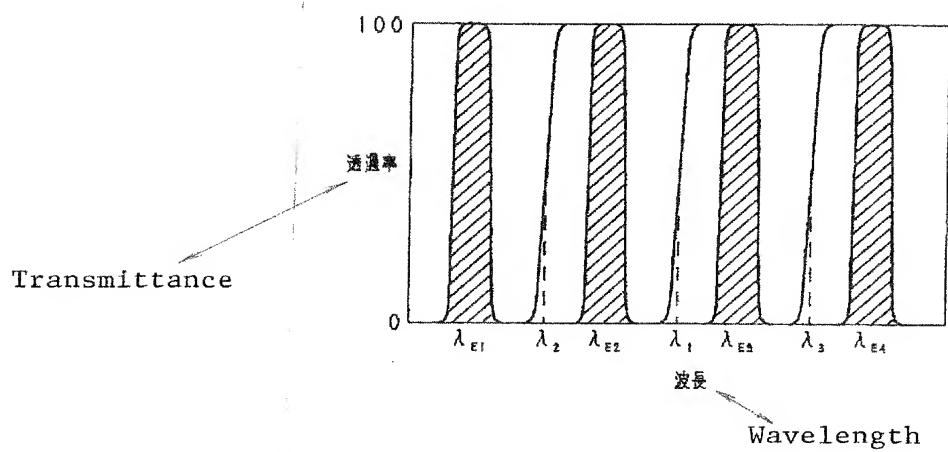
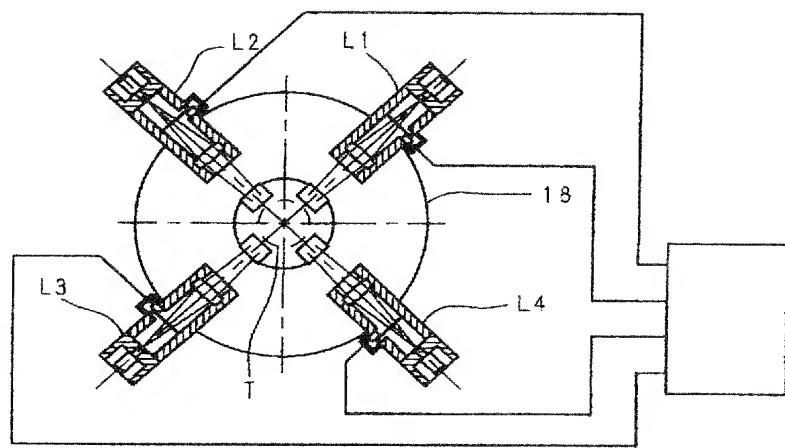


FIG. 11

【図 1 1】



【図 1 2】

FIG. 12

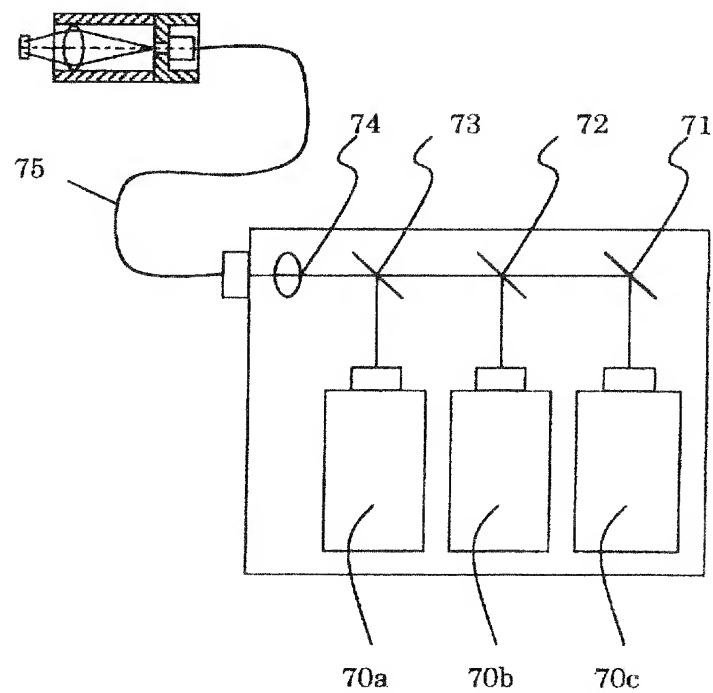


FIG. 13

【図13】

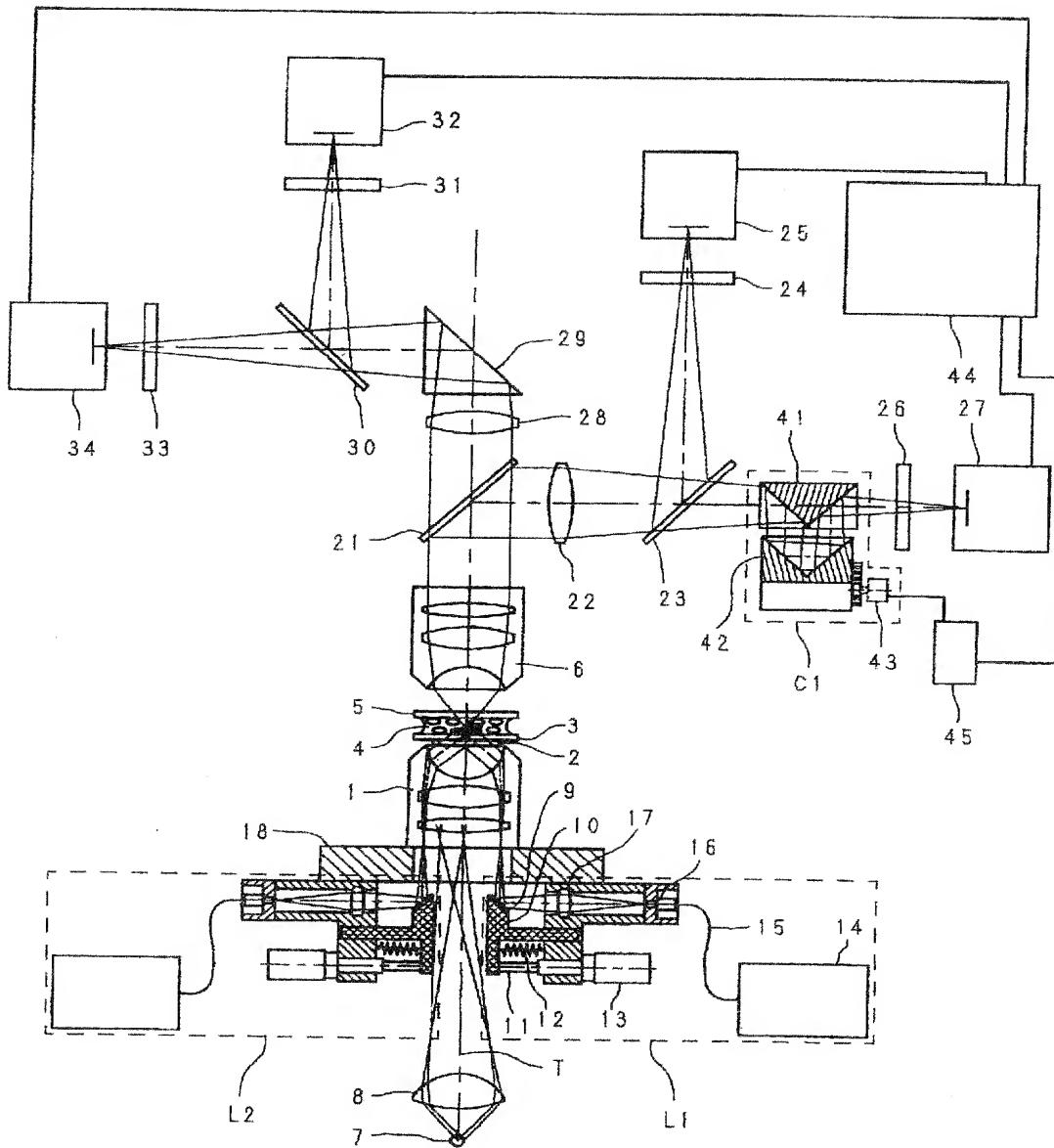


FIG. 14

【図14】

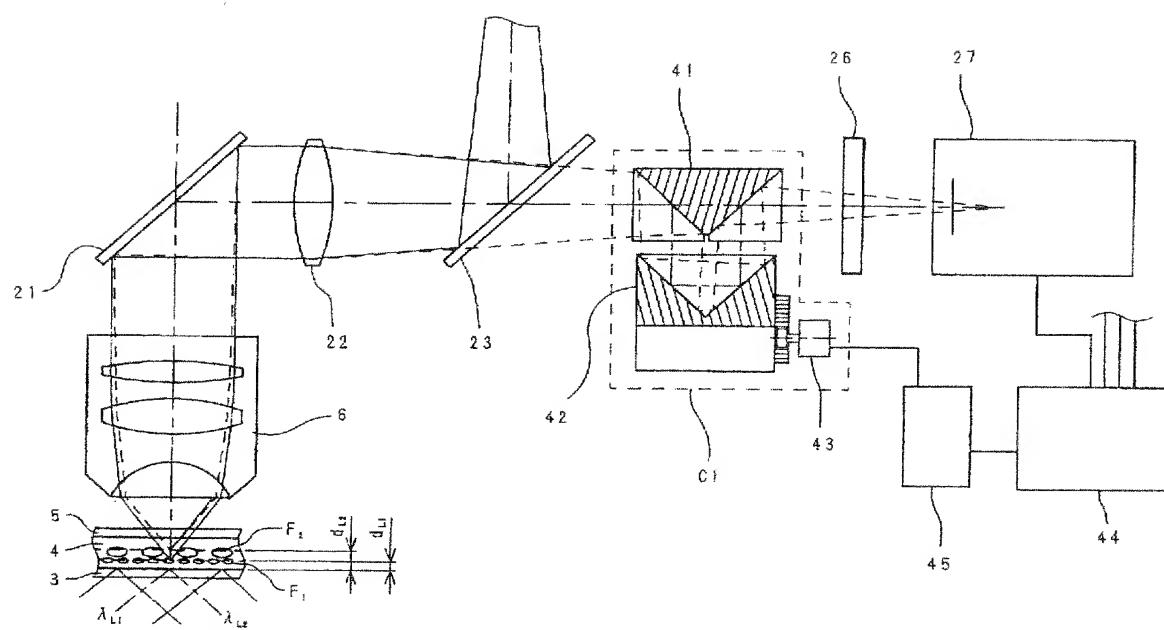


FIG. 15

【図15】

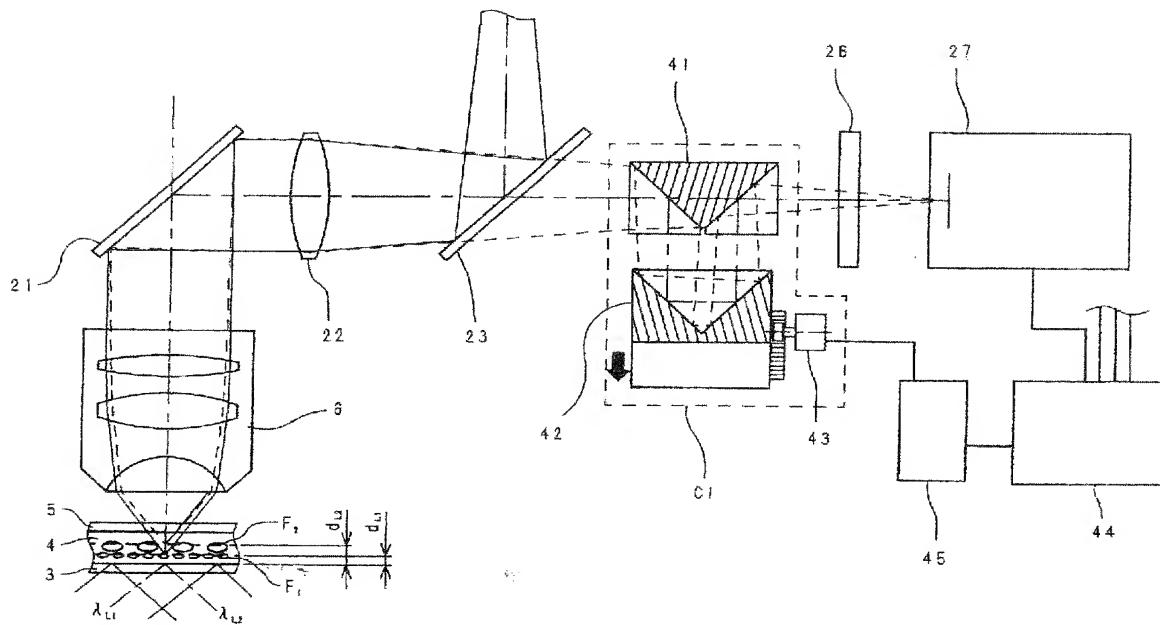
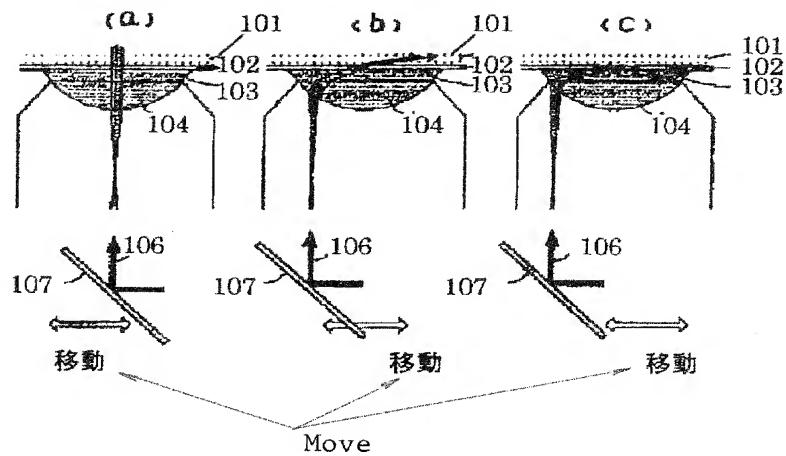


FIG. 16

【図16】



【図17】

FIG. 17

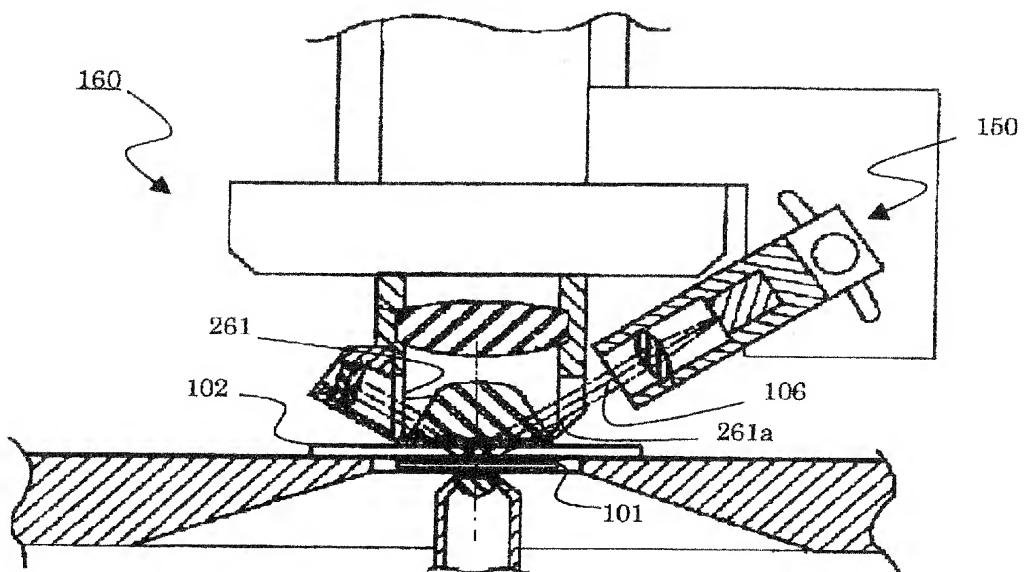
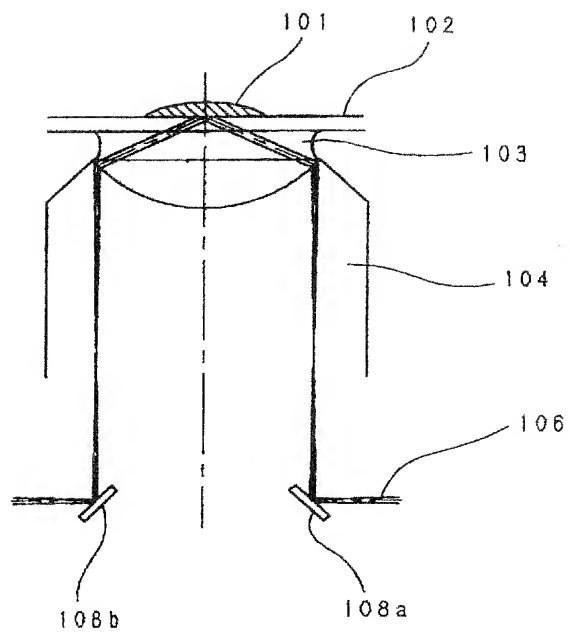


FIG. 18

【図18】



[Document] ABSTRACT

[Abstract]

[Object] To provide a total internal reflection fluorescence microscope which enables an objective lens having a magnification of lower than 60 times to achieve total internal reflection fluorescence observation, and which is compact and does not compress the operation space of a specimen with a superior operation property.

[Means for Achieving the Object] There is provided a total internal reflection fluorescence microscope comprising: a condenser lens for transmission illuminating 1 which is disposed in a position facing an objective lens 6 via a specimen 4 and which has a numerical aperture that makes possible total internal reflection illumination; a reflective mirror 9 which is movably disposed in the vicinity of an outermost part of an optical path of a transmitted illuminative light, closer to an optical source 7 side rather than the condenser lens 1 and which reflects a laser beam to introduce the laser beam on the condenser lens 1 side; a laser introductory optical path which allows the laser beam to be incident upon the reflective mirror 9 from a direction crossing the optical path of the transmitted illuminative light substantially at right angles; and a mirror holding section which moves the reflective mirror 9 in a direction parallel to the laser introductory optical path, wherein the laser introductory optical path comprising: a fiber 15 which transmits the laser beam output from a laser oscillation unit 14; and a condensing lens 17 which converts a divergent ray

emitted from an emission end of the fiber 15 into a convergent ray to condense the ray in the vicinity of a front focal position of the condenser lens 1 via the reflective mirror 9.

[Elected Figure] FIG. 1